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DEVELOPMENT OF FIELD APPLIED DDT

K. H. Sweeny, et al

Envirogenics Systems Company

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27 Abstract

Laboratory studies were carried out as a part of initial development of a concept of controlled destruction of field applied DDT pesticide. Copper catalyzed aluminum reductant was shown to degrade DDT in 24 hr at 25°C and 4 hr at 40°C without forming DDE. Copper catalyzed iron required a week to reduce DDT at 25°C and 8 hr at 40°C. Acidity for field degradation of DDT can be supplied by solid acids such as sulfamic, oxalic, or citric. An integrated degradable particle was demonstrated by a 5 μm reductant particle overlaid with sulfamic acid and coated with DDT. Only moisture is needed to initiate decomposition. In a demonstration 98.4% of the DDT was destroyed in 6 days and 99.8% in 2 weeks at 25°C. Product TTTB is 50-fold less fat soluble than DDT and nearly insoluble in water. Product DDEt is 20-fold more soluble than DDT in water. The vapor pressure of DDEt is about 80-fold greater than DDT. Exposure of fathead minnows, bluegills and rainbow trout to water saturated with DDEt (.05 ppm) or TTTB (.001 ppm) produced no acute toxic effects. The TLm ofDDEt to Daphnia is about 35 ppb. Long term chronic exposure of fathead minnows to DDEt saturated waters showed no effect on adult growth and survival, egg production or hatchability. Growth and survival of freshly hatched fry were affected by DDEt above about .006 ppm. No effect on fathead minnow adult or fry growth and survival, egg production or hatchability was shown by TTTB saturated water. Nearly mature fathead minnows consumed 10 mg/kg body weight/day of DDEt or 980 mg/kg body weight/day of TTTB in food without apparent deleterious effect. ~~(Sweeny -- Envirogenics)~~ (EPA)

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DEVELOPMENT OF FIELD APPLIED DDT

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ABSTRACT

Laboratory studies were carried out as a part of initial development of a concept of controlled destruction of field applied DDT* pesticide.

Copper-catalyzed aluminum reductant was shown to degrade DDT in 24 hrs at 25°C and 4 hrs at 40°C, without forming DDE. Copper-catalyzed iron required a week to reduce DDT at 25°C and 8 hrs at 40°C.

Acidity for field degradation of DDT can be supplied by solid acids such as sulfamic, oxalic, or citric. An integrated degradable particle was demonstrated by a 5 µm reductant particle overlaid with sulfamic acid and coated with DDT. Only moisture is needed to initiate decomposition. In a demonstration, 98.4% of the DDT was destroyed in 6 days and 99.8% in 2 weeks at 25°C.

Product TTTB is 50-fold less fat-soluble than DDT, and nearly insoluble in water. Product DDEt is 20-fold more soluble than DDT in water. The vapor pressure of DDEt is about 80-fold greater than DDT.

Exposure of fathead minnows, bluegills, and rainbow trout to water saturated with DDEt (.05 ppm) or TTTB ($\leq$.001 ppm) produced no acute toxic effects. The TLm of DDEt to Daphnia is about 35 ppb.

Long-term chronic exposure of fathead minnows to DDEt-saturated waters showed no effect on adult growth and survival, egg production, or hatchability. Growth and survival of freshly-hatched fry were affected by DDEt above about .006 ppm.

No effect on fathead minnow adult or fry growth and survival, egg production, or hatchability was shown by TTTB-saturated water.

Nearly mature fathead minnows consumed 10 mg/kg body weight/day of DDEt or 980 mg/kg body weight/day of TTTB in food without apparent deleterious effect.

This report was submitted in fulfillment of Contract 14-12-922 under the sponsorship of the Water Quality Office, Environmental Protection Agency.

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*See Glossary for chemical formulas and toxicological definitions.

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SECTION I

CONCLUSIONS

- Demonstration of the production and effectiveness of an integrated self-destructing pesticide particle was provided when 5 μm copper-catalyzed zinc particles were overlaid with sulfamic acid and the whole overlaid with DDT. Only moisture need be added for reaction, and the addition of a slowly dissolving or permeating membrane between the acid and DDT layer will give the desired delayed destruction. The integral particle is of such size that it can be dispersed by spraying or dusting. In one test, 98.4% of the DDT was destroyed in 6 days at 23-25°C, while in other tests an average of 99.8% of the DDT was destroyed in about 2 weeks at 23-25°C.

- Tests of the effect of time on the reduction of DDT by copper-catalyzed aluminum at 23-25°C showed that the DDT was essentially completely consumed in 24 hrs ($<$0.2-0.3% DDT) with no DDE as a product. Principal products were DDA and TTTB in nearly equal quantities. When the temperature was raised to 40°C, essentially complete degradation of DDT was obtained in 4 hrs, with the principal products being 2 1/2 - 3 parts TTTB to 1 part DDA. When iron catalyzed with copper was used as a reductant, the DDT was slowly consumed over a week at 25°C, with about 80% being destroyed in the first 24 hrs. The principal product was TTTB, with DDA occurring only in trace quantities. Increasing the reaction temperature to 40°C resulted in essentially complete reduction in 8 hrs; again TTTB was the principal degradation product.

- An alloy of aluminum and copper (5.4% Cu) was found to give essentially the same results as a surface-deposited copper catalyst in reducing DDT when a strong acid was used to provide the requisite acidity, although no reaction was shown with weak acetic acid. A series of metal couples were examined in an effort to find an improved catalyst for the Al-, Fe-, or Zn-catalyzed reduction of DDT. The transition and neighboring group metals examined were not as effective as copper in catalyzing the reduction of DDT.

- Practical field application of a self-destructing form of DDT would require the use of a solid-form acid to supply the requisite acidity in situ. Tests with copper-catalyzed aluminum reductant revealed that oxalic, sulfamic, tartaric and citric acids were effective, while only the oxalic and sulfamic acids were effective with aluminum-copper alloy reductant. Copper-catalyzed iron reductant was effective with sulfamic acid, while oxalic acid was intermediate in effectiveness. Zinc-copper reduction of DDT could be achieved with either sulfamic or citric acids.

- A large excess of reductant, ranging up to 5 parts reductant/part DDT or DDD was found effective in producing minimal DDD, but was not effective in reductively degrading DDE.

- Exposure of fathead minnows, bluegills, and rainbow trout to water saturated with DDEt or TTTB (concentrations respectively 0.05 and ≤ 0.001 ppm) resulted in no deaths or untoward behavior in 96-hr acute toxicity tests.

- Long-term chronic exposure of fathead minnows to waters saturated with TTTB appears to have no deleterious effect on adult growth and survival, egg production, egg hatchability, or the growth and survival of freshly-hatched minnow fry.

- Long-term chronic toxicity tests with fathead minnows showed that water saturated with DDEt had no deleterious effect on the survival and growth rate, egg production, and egg hatchability. However, freshly-hatched minnow fry survived poorly in DDEt-saturated water; the maximum concentration of DDEt which causes no effect on the fry appears to be about 0.006 ppm.

- Nearly mature fathead minnows can consume DDEt with their diet at a rate of 10 mg per kilogram body weight per day and TTTB at a rate of 980 mg per kilogram body weight per day without effect on growth, survival, and egg production. These dosages may not have any effect upon hatchability of eggs, but due to insufficient data it was not possible to ascertain the effects of DDEt- or TTTB-laden food on egg hatchability or larval growth and survival.

- DDEt, the principal product of zinc reduction of DDT, was found to be soluble in water to the extent of about 50-70 ppb, or about 20-fold greater than DDT. The solubility of TTTB, the product of catalyzed aluminum or iron degradation of DDT, however, was too low to be measured—1 ppb or lower. TTTB was found to be about 50-fold less soluble than DDT in a fat (triolein), while DDEt was about 2-1/2 times more soluble than DDT in the same medium.

- The vapor pressure of DDEt was determined to be about 80-fold greater than DDT at 23-25°C. A calculation shows that the rate of evaporative loss of DDT from an acre of glass would be one pound per 830 days, while the DDEt produced from one pound of DDT would last only 9 days.

SECTION II

RECOMMENDATIONS

The practicality of the concept of controlled degradation of field-applied DDT was believed amply demonstrated by the degradation of an integral particle containing the reductant and requisite acidity, overlaid with DDT - requiring only moisture to initiate decomposition. Further application of this important concept to the control of the persistence of chlorinated hydrocarbon pesticides is strongly recommended. Recommended activities include:

- Development of a controlled delay technique to permit pest control action for a stated period prior to the initiation of degradation.
- Tests to assure the pest control effectiveness of the integrated particle overlaid with DDT, and to demonstrate lack of phytotoxic effects.
- Studies to ascertain the most effective means for dissemination of the controlled-degrading form of DDT.
- Small-scale field tests of the controlled-persistence DDT, to establish the overall effectiveness, safety, cost, application hardware requirements, crop compatibility, soil residual agents, and reductant metal contamination implications.
- Modification of the concept for application to other field-applied chlorinated hydrocarbon pesticides, such as toxaphene, or heptachlor, including necessary toxic testing of degradation products.

Although the process leading to the formation of TTTB (Al• Cu or Fe• Cu reduction) appears safe to the fish tested, the effect of these degradation products on the shells of fish-eating or raptorial birds should be examined in suitable models in order to establish whether the "thin-shell" syndrome is exhibited.

SECTION III

INTRODUCTION

The objective of this study has been the development of a system for field-applied DDT which will degrade to a form harmless to life forms after a suitable delay to allow for pest control action. Thus, the concept is to provide a system having controlled persistence for chlorinated hydrocarbon type pesticides. The feasibility of the concept was shown in earlier studies on Contract 14-12-596 (Reference 1).

Two basic areas were investigated in these studies. In the first, the further development of the concept of degradation of a particle of DDT under simulated field conditions was carried out. In the second, the acute and long-term (chronic) toxicity of the two principal products of the reductive degradation of DDT, DDEt, and TTTB, were tested with appropriate fish species.

The earlier feasibility studies (Reference 1) had shown that DDT could readily be reductively degraded by either of two basic methods. In one, the reduction by zinc led to the formation of DDEt, in which all three aliphatic chlorines have been removed. In the second, treatment with aluminum produced a large molecule, TTTB, by reductively condensing two DDT molecules with the elimination of one atom of chlorine per DDT. Both reductive reactions were found to be catalyzed by small amounts of copper metal. In the studies to be described in this report, the reductive degradation of DDT has been examined in some detail. Particular emphasis has been placed on the use of catalyzed aluminum and catalyzed iron reductants, since the addition of these metals to the soil would appear to offer little environmental problem. Excessive zinc, on the other hand, could cause toxic problems to fish, although no problems were believed attendant to the use of zinc reductant at the levels employed in this study. The effect of continued metal application from repeated applications would require additional analysis.

Important to the practical application of this concept of controlled degradation of DDT is the development of a means for assuring a high probability of the degradation reaction taking place at the designated time. The achievement of reliable reaction conditions was believed to be demonstrated by studies with an integrated particle, in which a micron-sized catalyzed reductant particle was coated with a solid acid to provide the requisite pH for reaction, and the DDT was overlaid on the composite particle. Upon the addition of moisture, the reductive reaction occurred. Increased probability of reaction because of the close proximity of reactants, reduced reagent requirements in order to insure complete reaction, and less possibility of phytotoxic damage are some of the advantages of the integral particle concept. Since the DDT is on the outside of the particle in unaltered form, the effectiveness of the agent for pest control should not be diminished. This 5-10 micron particle would only require a time-delay membrane between from → Optimum reaction conditions and

the DDT layer and the solid acid-reductant particle to yield the desired controlled persistence pesticide system. Particles of this size can be readily sprayed with conventional equipment.

The development of the integral particle concept has required an evaluation of a variety of acids which might be used in practical systems. A study of possible improved catalysts was also made.

were studied

A second phase of the effort involved the characterization of the principal reaction products of reductive degradation of DDT. Product studies included water solubility, vapor pressure of pure DDEt, the hydrolytic stability, and resistance to further reduction.

The toxic testing to fish of the two principal degradation products of reductive decomposition of DDT, DDEt and TTTB, consisted of the examination of the acute toxicity to the fathead minnow and from this study, to carry out long-term chronic studies on the same fish. The long-term effect on survival, egg production, hatch rate, and survival and growth of fry was examined. The acute toxicity to rainbow trout and bluegills was also briefly studied. The acute and chronic bioassay tests were performed using EPA recommended protocols.

SECTION IV

DEVELOPMENT OF OPTIMAL DDT DEGRADATION REACTION CONDITIONS

The study of the feasibility of controlled degradation of DDT carried out under Contract 14-12-596 (Reference 1), disclosed two basic methods for the reductive degradation of DDT. These techniques were selected from a consideration of the following criteria:

- Degradation to proceed to the greatest extent possible, removing a significant amount of chlorine from the molecule or making the molecule biologically inert. The products DDE and DDD were to be avoided if possible. The major products should be harmless to life forms, whether mammalian, fish, or bird life.

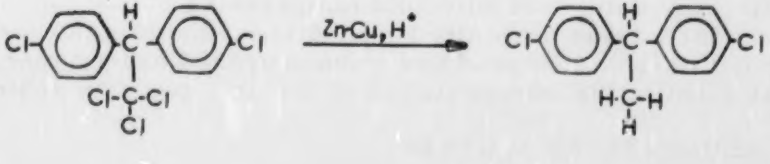
- The degradative reaction should proceed to a substantially complete destruction of DDT at ambient temperature (~25°C) in periods of a week or less.

- The reaction should be capable of being carried out in both soil and water, so that detoxification of both of these media may be achieved.

- The degradative technique should be economically feasible, and should not employ difficult to obtain materials.

- The catalyst or degradative materials used should not result in the introduction of harmful materials into the environment.

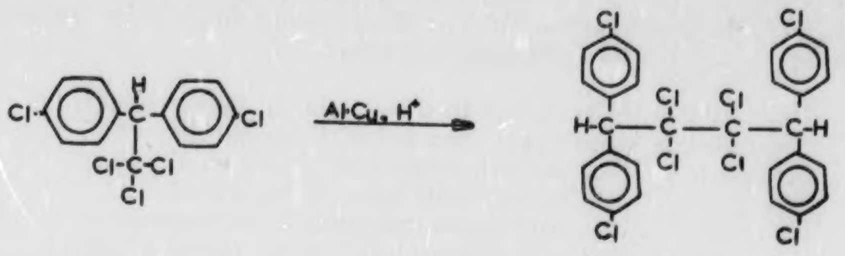
In the first of these methods, catalyzed zinc reductant led to the removal of the three aliphatic chlorines, leaving DDET as the principal product.



DDT

DDEt

While this method proceeded smoothly to the stated product, some zinc ion was also produced. However, the analysis given in Reference 1 suggests that the zinc ion concentration will be so small so as not to be a problem, unless repeated applications lead to excessive metal. In the second technique, catalyzed aluminum, or possibly iron, can be used as the reductant. In this case, a large, apparently insoluble molecule is formed which is presumed to be biologically inert. This species is formed by reductively coupling two DDT molecules:



DDT

TTTB

Since the presence of small quantities of aluminum or iron in runoff waters would appear to offer no environmental hazard, this technique was favored. Decreased reductant requirements also suggested that the method might be more economical in operation, as well as providing less metal for absorption and build-up in the soil on repeated applications.

ANALYTICAL TECHNIQUE

Principal analyses in this study were made using gas chromatography. While the basic technique was described in Reference 1, some differences from the previous study were made in an effort to improve accuracy and reliability. The analyses were made with a Perkin-Elmer Model 990 Gas Chromatograph, equipped with dual flame ionization and Ni 63 electron capture detectors; the flame ionization mode was employed for most of the studies. Glass chromatographic columns 6 ft long and 4 mm ID packed with 2% SE-30 (methyl silicone gum rubber) on silanized diatomaceous earth were used. Standard operating conditions were nitrogen carrier gas at 80 cc/min, injection block temperature 165°C, column temperature programmed from 140° to 240°C at 6°/min, and a detector manifold temperature of 220°C. The sample volume injected was normally 1 μl. Standardization curves for all decomposition/reaction products were prepared and regularly checked so that quantitative interpretation of the data could be achieved.

CATALYZED ALUMINUM REDUCTION

Reaction at 23-25°C

Earlier tests in which copper-catalyzed aluminum reductant was employed for degrading DDT indicated that substantially complete reaction was achieved in 2 to 4 days at 25°C (Reference 1). However, the rate of reaction had not been further defined. A series of tests was therefore undertaken in an effort to define the time required to carry out this reaction under both ordinary ambient conditions (~25°C) and simulated summer weather temperature cycles. In the initial test, a reaction was carried out for 25 hours at 25°C. In this test, 1 g of CP aluminum powder was added to 1 g of DDT in 20 ml of acetone, 1 meq of copper ion was added to form the catalyst couple, and the solution was acidified with 10 ml of 1.5 N acetic or sulfuric acid. At the conclusion of the test, the acetone-soluble products were filtered off for gas chromatographic analysis. The remaining mass of unreacted aluminum, copper catalyst, and TTTB was extracted with hot benzene to remove the TTTB. The TTTB was collected and weighed. The TTTB collected represented 33.2% of the weight of DDT when acetic acid was used, and 37.7% when sulfuric acid was employed. The benzene extract had a melting point in each case of 270°C (uncorrected), which is the same as that obtained previously with TTTB.

A second series was initiated in which the reaction was allowed to proceed for several different times at 25°C; acetic acid was used in these tests. Initially, the results showed that when reaction had occurred (i.e., nearly all of the DDT was consumed), a balance of products accounting for only about one half of the initial DDT was obtained. Although the possibility that a product not responding to the gas chromatograph, such as the 1,1,4,4-tetra(p-chlorophenyl)-2,3-dichlorobutene-2 product (TTDB) identified by Mosier, Guenzi and Miller on photochemical decomposition of DDT (Reference 2), might account for the poor balance, an additional product was believed present. Analysis of the acetone-soluble fraction revealed the presence of DDA, which does not respond to the gas chromatograph unless a derivative (e.g., methyl or silyl ester) is formed. Extraction, precipitation, and recrystallization of the acetone extract revealed a substantial amount of DDA in the samples. The identity of the product was confirmed by melting point, including mixed melting point tests with known material, and infrared analyses. The results of tests after reaction for 8, 24, 48, 96, and 168 hrs reaction at 23-25°C are shown in Table 1.

TABLE 1

EFFECT OF TIME ON Al·Cu REDUCTION OF DDT* AT 23-25°C

Component	Analysis**, %, after Reaction, hrs				
	8	24	48	96	168
DDEt	0.2	0.6	0.9	2.0	0.9
DBP	1.3	6.9	5.3	5.6	8.1
DBH	2.1	4.8	9.0	6.6	7.6
DDD	1.3	2.8	3.4	2.1	3.2
DDT	68.0	0.3	0.3	0.2	0.2
TTTB	17.0	36.6	40.8	50.0	39.1
DDA	10.9	38.0	34.6	26.6	34.0
Minor Components	1.0	1.1	1.4	1.4	1.5
Balance	101.8	91.1	95.7	94.5	94.6

*1 g of DDT in 20 ml acetone, 1 g of Al powder catalyzed with 1 meq Cu ion was added and the mix acidified with 10 ml 1.5 N acetic acid. Reactants were stirred with a magnetic stirrer.

**Calculated as equivalent % DDT.

These results have been calculated on the basis of the equivalent percentage of DDT reacting, so that a material balance can be made.

The results show clearly that for 24 hrs reaction (or longer), the DDT is nearly completely destroyed (0.2 - 0.3% remaining), with TTTB and DDA as principal products. Since DDA is a principal product of mammalian metabolism of DDT, it was considered a safe product. DDE was not found and minimal amounts of DDD were shown. Moderate amounts of DBP and DBH, which are also known metabolites of DDT, were also determined (Reference 3).

The data shown in Table 1 have been examined by standard reaction kinetic procedures. Although the test times provided practical data on the time required to effectively decompose DDT, the time periods were not satisfactory to determine the reaction rate for the disappearance of DDT. An attempt was made to determine the reaction order for the appearance of TTTB and DDA. Although the data for TTTB appearance fit a 1st order plot reasonably well at 24, 48 and 96 hrs, the 8-hr and 168-hr points deviate significantly. The rate of appearance of DDA likewise can be reasonably represented by 1st or 2nd order kinetics after 24, 48 or 96 hrs, but the initial (8-hr) and final (168-hr) points deviate sharply. The existence of several minor products, as well as the two major products, suggests that either parallel or consecutive reactions are probably being obtained so that a clear-cut kinetic analysis of the data would be difficult, and outside the scope of these studies.

Reaction at 40°C

The extent of reaction after 1, 2, 4, 8, and 24 hrs reaction at 40°C was also determined. This test is a simulation of conditions which might exist in the summer in southern agricultural lands. Temperatures as high as 45°C are reported for Georgia soils (Reference 4). These results follow in Table 2.

TABLE 2

EFFECT OF TIME ON Al• Cu REDUCTION OF DDT* AT 40°C

Component	Analysis**, %, after Reaction, hrs				
	1	2	4	8	24
DDEt	0.1	0.3	1.3	1.6	1.7
DBP	1.3	1.6	4.0	5.4	5.6
DBH	-	0.4	1.5	5.2	6.1
DDD	0.9	1.6	4.3	4.2	3.9
DDT	72.0	62.0	0.6	0.7	0.9
TTTB	14.3	18.9	58.3	54.3	55.5
DDA	8.2	11.6	19.7	21.0	19.7
Minor Components	1.0	1.2	6.7	2.4	2.4
Balance	97.9	97.6	96.4	94.8	95.8

*Conditions, except for temperature, same as in Table 1.

**Calculated as equivalent % DDT.

These data indicate that substantially all of the DDT has reacted after 4 hrs at 40°, comparable to 24 hrs at 25°C. Again, the sum of the TTTB and DDA is approximately 75% after 4 hrs or more reaction, but the product is predominantly TTTB at the 40°C reaction temperature.

The data in Table 2 could not be represented by any simple kinetic rate equation. Both the formation of TTTB and of DDA were examined.

Use of Sulfuric or Acetic Acids

There appears to be a further factor involved in the reaction related to the acid employed. In one set of experiments, the reaction of DDT with Al-Cu reductant was carried out in parallel using both acetic and sulfuric acids as the acidifiers. In these tests, 1 g of DDT in 20 ml of acetone was treated with 1 g of aluminum powder catalyzed by the addition of 1 meq of copper. The acidity was provided by adding 10 ml of 1.5 N acid. The weight of TTTB and DDA, after 24 and 141 hrs reaction at 25°C is shown in Table 3.

TABLE 3

EFFECT OF ACID TYPE ON YIELD OF TTTB FROM Al-Cu REDUCTION OF DDT

	TTTB*, %	DDA,* %
24 hr Reaction		
Sulfuric Acid	59.8	15.5
Acetic Acid	32.4	33.6
141 hr Reaction		
Sulfuric Acid	48.2	10.2
Acetic Acid	42.1	27.7

*Calculated as equivalent % DDT.

These results show that the amount of TTTB produced was greater when sulfuric acid was employed than for those runs in which the acidity was provided by acetic acid, and that more DDA was obtained when acetic acid was used. The effect of acid will be explored further in another section of this report.

Reduction with Al-Cu Alloy

It would be important to determine whether a finely divided aluminum-copper alloy powder could be used in place of the freshly-prepared couple currently employed as a reductant, since the alloy would represent a practical means for using copper-catalyzed aluminum reductant. A sample of an aluminum-copper alloy powder containing 5.4% copper (particle size: 90% passing 44-micron sieve) was supplied for this analysis by Reynolds Metals Company.

Two determinations were made. In each, 1 g of alloy was reacted with 1 g of DDT in 20 ml of acetone. However, in one sample the acidity was supplied by 10 ml of 1.5 N sulfuric acid; in the other 10 ml of 1.5 acetic acid. In both cases the reaction was carried out for 24 hrs at ambient temperature (23-25°C). The results of TTTB and DDA precipitation, and gas chromatography of the acetone-soluble fraction, follow in Table 4.

TABLE 4

REDUCTION OF DDT WITH Al• Cu ALLOY*

Component	Analysis**, after Reaction for 24 hrs at 23-25°C	
	Sulfuric Acid	Acetic Acid
TTTB	72.7	0.6
DDA	6.6	-
DDEt	2.8	<0.1
DBP	1.3	1.0
DDMU	-	0.2
DDMS	-	<0.1
?	1.3	0.2
DDD	1.0	0.4
DDT	0.4	100.0
Balance	86.1	102.4

*1 g of DDT in 20 ml acetone, 1 g Al• Cu powder, and acidified with 10 ml of 1.5 N acid. Reactants stirred with magnetic stirrer.

**Calculated as equivalent % DDT.

A clear difference is shown between these samples. The reaction carried out in an acetic acid medium has clearly not proceeded to a useful state. However, when sulfuric acid was employed, the reaction proceeded largely to TTTB (72.7% of DDT converted to TTTB). The result with sulfuric acid is comparable to that obtained when a freshly-formed Al• Cu couple is employed.

CATALYZED IRON REDUCTION

Reaction at 23-25°C

It was of interest to examine the rate and extent of reaction when DDT was reduced by catalyzed iron reductant. Although some data had been reported for isolated tests of this system, a series to show the extent of reaction with reaction time had not been carried out. In this series, 1 g of DDT was reacted with 1 g of powdered iron to which 1 meq of copper ion had been added to form the couple. The solution (2:1 acetone-water) was O. 5 N in acetic acid. The results of analyses on these samples, expressed as equivalent DDT percent, are given in Table 5.

TABLE 5

EFFECT OF TIME ON Fe• Cu REDUCTION* OF DDT AT 25°C

Component	Analysis**, %, after Reaction, hrs			
	[illegible]	48	96	168
DDEt	1. 1	2. 4	1. 3	1. 2
DBP	0. 9	1. 7	0. 8	0. 8
DDE	1. 7	2. 0	1. 9	1. 9
DDD	12. 9	15. 5	15. 1	14. 3
DDT	16. 7	9. 9	7. 2	2. 4
TTTB	59. 6	64. 3	63. 5	69. 3
Balance	92. 9	95. 8	89. 8	89. 9

[*] 1 g of DDT in 20 ml acetone, 1 g of CP powdered iron catalyzed with copper ion was added and the mix was acidified with 10 ml of 1. 5 N acetic acid. The reactants were stirred with a blade stirrer.

[**] Expressed as equivalent % DDT.

These results indicate a first order consumption of DDT with TTTB as the principal product. The iron system does not appear to be as rapid as the copper-catalyzed aluminum or zinc systems. Only trace quantities of DDA were found when copper-catalyzed iron reductant was used, in contrast to the results with the Al• Cu system in which TTTB and DDA were found in nearly equal quantities.

Reaction at 40°C

The effect of increasing the reaction temperature to 40°C, simulating extreme summer soil conditions, was also noted. The results of analyses follow in Table 6.

TABLE 6

EFFECT OF TIME ON Fe• Cu REDUCTION* OF DDT AT 40°C

Component	Analysis**, %, after Reaction, hrs 4	8	17	24	72
DDEt	0.9	1.0	1.5	1.2	4.8
DDD	10.4	10.1	7.3	14.9	3.1
DDT	9.5	0.4	0.4	0.0	0.2
TTTB	77.7	93.9	93.7	88.4	78.0
Minor Components (DBP, DDMS, DDMU, etc.)	2.0	1.8	2.1	1.8	2.9
Balance	100.5	107.2	105.0	106.3	89.0

*Conditions, except temperature, same as Table 5.

** Converted to equivalent % DDT from which component was produced.

These results indicate that the increase in temperature from 25° to 40°C produced a striking increase in the rate of reaction, since essentially complete reaction was obtained in 8 hrs at 40° while 168 hrs at 25° was required for an equivalent reaction. It should be noted that the copper-catalyzed iron reduction of DDT leads to nearly complete conversion of DDT to the insoluble product TTTB; however, it appears that the TTTB may be partially consumed at reaction periods greater than 17 hrs. The balance after 72 hrs reaction may indicate the formation of a new product which does not respond to the gas chromatograph. An attempt to fit the data to kinetic rate equations was unsuccessful.

Use of Sulfuric or Sulfamic Acids

Following tests with the Al• Cu system described later in this section on the effect of acid type on the reaction, tests were carried out in which sulfuric and sulfamic acids were used for acidifying the reactants. The reaction was carried out in the same manner as described above. In either case the reaction mix was 0.5 N in acid. The results of tests are presented in Table 7.

TABLE 7

EFFECT OF ACID TYPE ON REDUCTION OF DDT BY Fe•Cu REDUCTANT*

Component		Analysis**, %, after 24 hr Reaction at 25°C	
	Acid:	Sulfuric	Sulfamic
DDEt		0.7	2.8
DBP		1.8	3.3
DDE		1.6	1.7
DDD		3.4	15.1
DDT		64.5	0.1
TTTB		10.5	64.7
	Balance	82.5	87.7

*1 g of DDT in 20 ml acetone and 1 g of CP powdered iron catalyzed with 1 meq of copper ion was added and the mix was acidified with 10 ml of 1.5 N acid. The reactants were stirred with a blade stirrer.

**Expressed as equivalent % DDT.

It is interesting to note that while a large portion of DDT was unreacted when sulfuric acid was employed, essentially complete consumption of the DDT was achieved with sulfamic acid, producing largely TTTB as a product. Negligible amounts of DDA were found.

CATALYZED ZINC REDUCTION

Consideration of the catalyzed zinc reductant system would require the use of minimum amounts of zinc, since excessive zinc ion is toxic to fish (Reference 1). A limited series of experiments was carried out in an effort to establish minimum zinc-to-DDT ratio for effective reduction. The previous tests (Reference 1) had employed equal weights of zinc and DDT (the theoretical zinc required to reduce 1 g of DDT to DDEt is 0.276 g) or a 2-1/2-fold excess over stoichiometry.

In these tests, from 0.1 to 3 g of Zn per g of DDT was used; the ratio of copper catalyzt to zinc was held constant at 1 meq copper ion added per g Zn.

In the new series, 20 ml of acetone containing 1 g DDT was added to the specified amount of zinc, the given amount of copper ion was added to form the Zn•Cu couple, and the solution was made 0.50 N in acetic acid. The reactions were carried out for 2 hrs at ambient temperature (23-25°C). The results of analyses are shown in Table 8.

TABLE 8

EFFECT OF AMOUNT OF Zn·Cu REDUCTANT ON REDUCTION OF DDT*

		Analyses**, %, after 2 hr Reaction at 25°C				
Component	g Zn/g DDT:	0.10	0.20	0.30	0.40	0.50
DDEt		5.4	12.2	21.6	29.5	34.8
DDMS		0.5	1.4	1.5	2.3	2.5
DDD		9.6	22.5	41.1	43.0	45.5
DDT		77.4	48.8	26.9	5.0	2.8
Minor Components (DBP, DMC, DDMU, etc.)		1.1	3.2	3.5	4.1	4.2
	Balance	98.2	90.9	95.3	84.3	90.4
Component	g Zn/g DDT:	0.75	1.0	1.5	2.0	3.0
DDEt		41.8	52.7	61.4	71.2	76.6
DDMS		4.8	7.1	6.2	6.2	7.8
DDD		40.2	30.0	21.8	8.1	3.5
DDT		1.1	-	-	-	-
Minor Components (DBP, DMC, DDMU, etc.)		4.6	4.6	4.9	4.4	3.6
	Balance	92.3	94.9	94.3	89.9	91.7

*1 g of DDT added in 20 ml acetone, followed by given amounts of CP zinc dust. Copper ion was added at the rate of 1 meq/g Zn dust and the mix was acidified with 10 ml 1.5 N acetic acid. The reactants were stirred with a magnetic stirrer.

**Converted to equivalent % DDT.

These results show a dramatic decrease in the DDD obtained, with an equivalent increase in DDEt production, as the ratio of zinc to DDT is increased. These results show that a large excess of reagent will force the reaction to the desired products. Indeed, it suggests that a reactor configuration in which a large excess of reagent is present (such as a packed bed) may be the preferred method of reaction for such applications as industrial waste treatment.

In an additional series of tests, the effect of changing the ratio of copper catalyst to zinc reductant was studied. These tests were carried out in the same manner as the previous series except that 1 g of zinc was used per g of DDT, and the amount of added copper ion was varied. These tests were also carried out for 2 hrs at ambient temperatures (25°C). The results follow in Table 9.

TABLE 9

EFFECT OF AMOUNT OF COPPER CATALYST ON REDUCTION OF DDT BY ZINC*

Component		Analysis**, %, after 2 hr Reaction at 25°C					
	Cu, meq:	0.25	0.50	1.0	1.5	2.0	5.0
DDEt		37.2	45.7	42.3	48.0	55.1	38.1
DDMS		8.7	6.0	5.6	3.7	7.2	5.2
DDD		32.5	33.1	34.3	31.6	23.0	38.8
DDT		-	-	-	-	-	-
Minor Components (DDMU, DBH, etc.)		5.0	4.4	4.8	5.0	4.8	4.2
	Balance	85.0	89.2	87.0	88.3	90.1	86.3

*Procedure same as used in Table 8, except that 1 g Zn dust was used and added copper ion was varied per table.

**Converted to equivalent % DDT.

These results do not show an identifiable effect on the reaction as the amount of catalyst is changed, except that the reaction at the extremes of catalyst (0.25 and 5.0 meq Cu/g Zn) appear to give a poorer yield of DDEt. The best DDEt production (and lowest DDD) was given at 2.0 meq Cu/g Zn.

In another test, a 1 g sample of DDT was reacted with 3 g of zinc dust to which 1 meq of copper ion was added. The data from this test indicates less conversion than was obtained when 3 meq of Cu was used with 3 g of zinc reductant.

TABLE 10

EFFECT OF VARYING Zn/Cu RATIO ON EXTENT OF REDUCTION OF DDT*

Component	Analysis**, %, after 2 hr Reaction at 25°C 3 g Zn, 1 meq Cu	 3 g Zn, 3 meq Cu***
DDEt	66.5	76.6
DDMS	11.2	7.8
DDD	12.1	3.5
DDT	-	-
Minor Components (DBP, DDMU, DMC)	5.9	3.6
Balance	95.7	91.7

*Procedure same as used in Table 8 and 9.

**Converted to equivalent % DDT.

***Repeated from Table 9 for comparison.

Zinc-Copper Alloy Reductant

The zinc-copper reductant is normally prepared by adding copper ion to slurried zinc powder, whereupon the copper ion precipitates onto the surface of the zinc powder by electrochemical replacement. It was of interest to determine whether similar efficacy of reduction could be achieved if a zinc-copper alloy were used, since the use of an atomized alloy powder might represent a practical means for preparation of the catalyzed zinc. The optimal ratio of surface-deposited copper appears to be about 1 meq/g zinc, which represents about 3% copper on a total particle basis. Samples were prepared by blending zinc dust and copper powder (3, 5, and 10%), placing the mix in a porcelain crucible, and heating the mixture in an electric furnace while flooding the furnace with nitrogen gas so as to reduce oxidation of the zinc. However, despite these precautions, some ZnO appeared to have been formed. The samples were heated to 875°C, well above the reported melting point of the alloys (Reference 5) of about 460° for the 3% alloy, 500° for the 5% composition, and 580° for the alloy containing 10% copper. The alloys were then cooled, and the pellets were reduced to a fine powder by filing. Iron from the file was removed magnetically. The samples were then reacted with DDT by the usual method: 1 g reductant was reacted with stirring in 20 ml acetone + 10 ml 1.5 N acetic acid with 1 g of DDT. The reaction was carried out for 26 hours at 25°C. The results are shown in Table 11.

TABLE 11

EFFICACY OF Zn-Cu ALLOY FOR REDUCTION OF DDT

		Analysis*, %, after 26 hr Reaction at 25°C				
Component	Sample:	3% Cu Alloy	5% Cu Alloy	10% Cu Alloy	Zn• Cu Couple	Zn Dust
DDEt		57.4	26.4	48.0	82.1	59.5
DDMU		2.8	2.1	3.3	3.8	5.9
DDMS		10.0	7.8	11.3	12.0	20.7
DDD		29.7	63.7	37.4	2.1	13.8
DDT		-	-	-	-	-
	Balance	99.9	100.0	100.0	100.0	99.9

*Converted to equivalent % DDT.

While all of the alloy samples were effective in destroying DDT, none was as effective as the freshly-deposited couple in forcing the reaction to the desired product DDEt while forming minimal amounts of DDD. It is of interest that the alloy sample containing the 3% copper appeared to be the best of the alloys, although it was not quite as good in this series as the uncatalyzed zinc.

EFFECT OF ACID TYPE ON DDT REDUCTION

The practical development of a controlled self-destructing form of DDT for use in the field requires the combination of reductant, requisite acid for the reaction, and a means for suitably delaying the degradation process. A careful consideration of the requirements suggests further that it would be desirable to employ a solid form of the acid since (1) dissemination would be simpler, (2) minimum acid would be required since it would be necessary to provide it only at the reaction site, and (3) possible phytotoxic effects from the acid could be avoided. It has been shown in earlier studies that reduction can be achieved by the Zn• Cu couple in the presence of acetic, sulfuric or hydrochloric acids, with the reaction proceeding best at a pH <4 (Reference 1). It remained to be established if other acids could be employed, and whether properties such as the solubility, acid strength (dissociation constant), etc., would have an effect on the extent of the degradation reaction. A series of eight solid state acids were examined and compared with acetic acid. The acids and some of their chemical and physical properties are shown in Table 12.

TABLE 12

CHARACTERISTICS OF CANDIDATE SOLID ACIDS

Acid	Formula	Cost $/lb	Dissociation Constant K_1	Dissociation Constant K_2	Dissociation Constant K_3	Solubility g/100 g H_2O at Ambient Temperatu
Sulfamic	$H_2N\ SO_2OH$	0.15	7.9×10^{-1}	-	-	14.7
Citric	$(COOH)CH_2C(OH)$- $(COOH)CH_2COOH$	0.30	8×10^{-4}	1.8×10^{-5}	4×10^{-6}	13.3
Oxalic	$(COOH)_2$	0.21	3.8×10^{-2}	4.9×10^{-5}	-	10.0
Fumaric	$(CH_2COOH)_2$	0.175	1×10^{-3}	3×10^{-5}	-	0.7
Maleic Anhydride	$(CHCO)_2O$	0.14	1.5×10^{-2}	1.3×10^{-6}	-	16.3
Suberic	$(CH_2)_6(COOH)_2$	-	3.0×10^{-5}	-	-	0.14
Tartaric	$(CHOHCOOH)_2$	0.415	9.6×10^{-4}	2.9×10^{-5}	-	120
Lactic	$CH_3CHOHCOOH$	0.275	1.4×10^{-4}	-	-	∞
Acetic[+]	CH_3COOH	0.09	1.8×10^{-5}	-	-	∞

[+] Not a solid acid; shown for comparison.

These acids were selected on the basis of the solubility range and dissociation constant, and all are common items of commerce.

The tests were made by adding 1 g of DDT in 20 ml acetone to a flask containing 1 g of zinc powder. The copper couple was then formed by adding with stirring 1 meq of copper ion in aqueous solution. To this suspension, 10 ml of water was added, and 15 millimoles of the acid was then added at the inception of the reaction. After two hours reaction at ambient temperature (23-25°C), the reactants were filtered and the solutions analyzed. The solutions were not analyzed for TTTB or DDA. The results of these analyses are presented in Table 13.

An examination of these results shown that effective reduction was achieved when sulfamic, citric, or acetic acid was used, and that little reduction was achieved when maleic anhydride was employed as the acid. Reduction was also achieved when the acidity was provided by oxalic, fumaric, or suberic acids, although the reaction did not proceed as far as when citric, acetic, or sulfamic acids were employed (lower DDEt, higher DDD percentage when oxalic acid, etc., used). In examining the basis for the observed efficacy of action of these acids, it may be noted that two of the less efficient acids, suberic and fumaric acids, are only sparingly soluble. The efficient acids show a range of dissociation constants. Sulfamic acid was the strongest acid employed, acetic the weakest, and citric acid was intermediate in acidity. The lack of product balance may indicate the formation of TTTB or DDA, for which no analysis was made.

Similar tests were carried out with the copper-catalyzed aluminum reduction of DDT. In these tests, 1 g of DDT in 20 ml acetone was added to a flask containing 1 g Al powder. The Cu couple was formed by adding, with stirring, 1 meq of aqueous Cu ion. To this suspension, 10 ml of water and 15 millimoles of the acid were added. The suspension was reacted for 24 hrs at ambient temperature (23-25°C), filtered and analyzed. The results of these analyses are presented in Table 14.

It is clear that most of the acids have given essentially complete reduction of the DDT. Only the suberic acid led to significant unreacted DDT. The small amount of DDT (0.3 - 0.7%) in the remaining flasks is believed to represent material splashed onto the flask wall where it was removed from the reaction. The greatest yield of the product TTTB was shown with oxalic, sulfamic, tartaric, and citric acids. The balance of products is poor and may represent at least in part DDA which was not analyzed for. These tests were carried out before DDA product was discovered. The procedure used was believed adequate to recover DDT and related products possibly trapped in the solids.

Since the alloy of aluminum and copper would appear to be the most practical way of carrying out a copper-catalyzed aluminum reduction of DDT in the field, a test of the efficacy of selected solid acids with this system was also desirable. Sulfamic, citric, and oxalic acids were examined, based on the above results. In these tests, 1 g of the Al•Cu

TABLE 13

EFFICACY OF SELECTED SOLID ACIDS IN REDUCTION[*] OF DDT BY Zn·Cu COUPLE

[**]Component		Analysis[***], %, after 2 hr Reaction at 25°C						
	Acid:	Oxalic	Maleic	Fumaric	Citric	Suberic	Sulfamic	Acetic[+]
DDEt		33.1	0.4	22.7	52.4	27.4	54.6	50.4
DBP		0.3	-	0.6	0.8	0.7	2.1	1.7
DDMS		4.2	0.4	3.2	5.2	4.5	4.0	6.2
DDD		42.8	2.2	56.0	29.1	38.1	21.2	26.0
DDT		-	91.3	-	-	0.8	-	-
	Balance[++]	80.4	94.3	82.5	87.5	71.5	81.9	84.3

[*] 1 g of DDT dissolved in 20 ml acetone and 1 g Zn catalyzed with 1 meq of Cu ion added. 10 ml of water added followed by 15 millimoles of solid acid. Mixture stirred with magnetic stirrer.

[**] Minor components not listed.

[***] Converted to equivalent % DDT

[+] Not a solid acid; shown for comparison.

[++] No analysis made for TTTB and DDA.

TABLE 14

EFFICACY OF SELECTED SOLID ACIDS IN REDUCTION OF DDT BY Al· Cu COUPLE*

**Component	Acid:	Analysis, %, after 24 hr Reaction at $25^{\circ}C$***			
		Lactic	Tartaric	Sulfamic	Citric
DDEt		1.3	2.8	4.2	2.4
DBP		3.8	1.1	3.4	1.6
?		3.4	-	-	-
DDE		1.3	1.3	0.9	1.2
DDD		3.8	4.1	2.4	3.8
DDT		0.3	0.3	0.1	0.3
TTTB		33.6	41.4	49.8	39.8
	Balance	47.5	51.0	60.8	49.1
		Maleic	Oxalic	Fumaric	Suberic
DDEt		1.1	3.2	2.3	0.8
DPB		14.1	1.4	2.6	4.5
?		-	-	2.8	5.6
DDE		1.4	2.2	1.1	0.9
DDD		2.4	4.4	4.1	3.2
DDT		0.7	1.6	0.7	19.2
TTTB		31.7	56.9	28.5	16.6
	Balance	51.4	69.7	42.1	50.8

*Procedure same as cited in Table 13 except aluminum powder rather than zinc dust used as reductant.

**Minor components (ca 1-2%) not listed.

***Calculated as equivalent DDT reacted.

alloy powder (particle size: 90% passing 44-μm sieve, 5.4 wt % copper), 1 g DDT, 20 ml acetone and 15 millimoles of the acid in 10 ml water were reacted for 24 hr at ambient temperature (25°C). The results of analyses of these samples follow:

TABLE 15

EFFICACY OF OXALIC, SULFAMIC AND CITRIC ACIDS IN Al·Cu ALLOY REDUCTION OF DDT*

Component	Analysis**, %, after 24 hr Reaction at 25°C		
	Oxalic	Sulfamic	Citric
DDEt	1.8	1.6	0.0
DDD	2.8	3.3	0.4
DDT	0.6	0.5	97.0
TTTB	73.7	69.2	0.6
DDA	not determined	4.9	-
Minor Components (DBP, DDMS, DDMU, etc.)	3.6	6.3	0.1
Balance	-	85.8	98.1

*Procedure same as cited in Tables 13 and 14 except that 1 g of Al·Cu alloy used instead of metal powder + copper ion catalyst.

**Converted to equivalent % DDT from which component was produced.

In this system, oxalic acid appears to be about as effective as sulfamic acid in degrading DDT, while the weaker, citric acid failed to degrade the DDT. Strong acids are apparently required to promote the reduction of DDT by Al·Cu alloy.

The effect of acid type on the reduction of DDT by copper-catalyzed iron was also considered. In this series of tests, 1 g of powdered iron was added to 1 g of DDT in 20 ml of acetone, and 1 meq of copper ion was added to form the catalyst couple. The solution was then acidified by adding 15 meq of the given acid in 10 ml of water. The results follow; a previous result in which aqueous acetic acid was used as the acid source is also given for comparison.

TABLE 16

EFFICACY OF SELECTED ACIDS IN THE REDUCTION OF DDT BY Fe• Cu COUPLE*

Component	Acid:	Analysis**, %, after 24 hr Reaction at 40°C			
		Sulfamic	Citric	Oxalic	Acetic
DDEt		2.8	-	0.6	1.2
DDD		14.8	0.8	3.6	14.9
DDT		0.0	99.0	60.5	0.0
TTTB		70.5	-	25.2	88.4
Minor Components (DBP, DDMS, DDMU)		2.7	0.2	1.8	1.8
Balance		90.8	100.0	91.7	106.3

*Procedure consisted of dissolving 1 g of DDT in 20 ml acetone, adding 1 g CP powdered iron powder and 1 meq copper ion catalyst, and then 10 ml of water. 15 millimoles of the acid was then added and the mixture was stirred with a blade stirrer.

**Converted to equivalent % DDT from which product was produced.

These results show that the sulfamic acid led to the effective reduction of DDT, while weaker citric acid was not effective in converting DDT to a less toxic form. Oxalic acid, which is nearly as strong as sulfamic acid, led to intermediate results. The comparative dissociation constants for the acids employed were shown in Table 12.

DEGRADATION OF INTEGRAL REDUCTANT-DDT-ACID PARTICLES

It has been shown that the best opportunity for degradation of DDT in the field should be achieved in a system whereby the reductant, DDT, and acid are kept in close proximity. An integral particle concept was outlined in an earlier report in which the reductant particle, coated with a solid acid and overlaid with the DDT, was considered to be a suitable means for carrying out the degradation reaction in the field. If this system, which could presumably be spray-applied or dusted, is shown practical, then the controlled delay could be introduced by applying a slowly-removed membrane between the solid acid-reductant and the DDT. Since the DDT surface is exposed, the pest-control action should be unaffected.

Tests of this concept were initiated and the results suggested that the tests were successful. Zn• Cu couple was prepared by placing 5 g of

5 μm powdered Zn in a flask and adding 20 ml acetone, followed by 5 ml of 1 N $CuCl_2$ solution to form the couple. The reactants were mixed for 5 min, filtered, washed with acetone and air dried.

In the initial series, one gram samples of the Zn• Cu couple were weighed into 50 ml beakers, and 10 ml of water was added. Then 5 millimoles of the solid acid (sulfamic acid or citric acid) was added and the water was evaporated from the samples. In some designated samples, 1 drop (~0. 02 ml) of Triton X-100 surfactant was added to aid dispersal. Some bubbling indicating a slight to moderate attack of the acid on zinc was noted. In an additional test, ethanol was used as the solvent for the acid; in this case the acid did not appear to attack the metal.

After the samples had dried, 0. 25 g DDT in 5 ml acetone was added and the acetone was evaporated. The samples were then placed on a 7 cm filter paper in a petri dish, a drop of water was added, and the dish covered. A drop of water was added daily to keep the paper moist. After 6 days, at ambient temperature, the samples were washed with acetone and analyzed. The results from some tests follow:

TABLE 17

ANALYSIS OF INTEGRAL Zn• Cu REDUCTANT-DDT-ACID PARTICLES AFTER REACTION

Component	Reductant: Acid: Triton X-100:	Analysis of Residue*, %, after 6 days at 24-26°C		
		Zn• Cu Sulfamic Ca 0. 02 ml	Zn•Cu Citric Ca 0. 02 ml**	Zn• Cu Citric
DDEt		75. 6	43. 9	0. 7
DDMU		0. 8	-	-
DDMS		16. 3	9. 1	-
DDE		-	-	tr
DDD		5. 7	28. 0	1. 2
DDT		1. 6	15. 8	98. 1
Sample Recovered, %		49	53	57

*All values converted to equivalent % DDT reacted.

**Ethanol solvent used for particle preparation.

The samples employing citric acid with water as a solvent had a sticky consistency and extensive reaction was not shown. However, the sample which had been acidified with sulfamic acid was a crumbly mass which could be readily separated into discrete particles. It is important to note that the DDT in this sample was nearly completely destroyed, the residue assaying 1.6% DDT. The residue was largely DDEt; the DDEt and DDMS fractions accounted for about 92% of the residue. Substantial decomposition of the sample acidified with citric acid and using ethanol as a solvent was also shown.

Quantitative recovery of the samples was not achieved, as noted by the values given for the percent of sample recovered. While handling difficulties, especially with the very sticky citric acid samples, may contribute to this problem, the unrecovered 50% in the sulfamic acid sample is believed due to other causes. The high evaporative loss of DDEt may be the reason for the apparently poor recovery.

Based upon these results, a second series of tests was carried out. A quantity of Zn• Cu couple was prepared and coated with either citric acid or sulfamic acid, and tests were carried out on aliquot portions of these batches.

The citric acid-coated particles were prepared by first slurrying 11 g of zinc dust in acetone, adding the requisite copper ion to form the couple, filtering and washing the couple, and drying under vacuum. Citric acid monohydrate (55 millimoles, 11.56 g) was then added to the flask, and 25 ml absolute ethanol was added to dissolve the citric acid. The solvent was then removed with difficulty in a rotary evaporator. The mix tended to be viscous and frothy, although no bubbling indicative of zinc reaction was noted. A weight loss equal to 9.4% (theoretical, 8.6%) of the citric acid monohydrate weight indicated that the acid was dehydrated in the vacuum drying process. The mass was then ground and mixed in an agate mortar, and 2.5 g DDT in an acetone solution was added, and the acetone was removed in the rotary evaporator. The sample was gently pulverized and split into 10 portions for testing. These samples were placed onto 11 cm filter papers in petri dishes, spread into a thin layer and moistened with a drop of water daily. A portion of the sample was withdrawn periodically, extracted five times with warm benzene and the solution analyzed by gas chromatography. The analyses of some of the samples are shown in Table 18.

TABLE 18

ANALYSES OF INTEGRAL DDT-Zn•Cu-CITRIC ACID PARTICLES AFTER REACTION

Component	Analysis, %, after time, hr, at 23-25°C*		
	0	20	70
DDEt	35.7	62.4	82.2
DDMU	2.6	2.8	2.9
DDMS	6.2	9.4	12.7
DDD	7.0	4.2	1.3
DDT	48.4	21.3	0.8

*Analyses calculated as equivalent amount of DDT reacted; analyses normalized to 100% because of differences in sample size.

An almost immediate reaction was noted and the zero time reaction shown was believed due to reaction during the handling of the samples following DDT application. It should be noted that DDD is being consumed during the reaction. The favorable conversion of the DDT using citric acid as the acidifier is somewhat surprising in view of the results of Table 17 and evidently represents an improved preparation.

Additional samples from the citric acid series (Table 18) were analyzed after 336 hrs, or 336 hr with 72 hr additional exposure after adding citric or acetic acid. These results are an indication of the completeness and reproducibility of the reaction. The results are shown in Table 19.

TABLE 19

ANALYSES OF INTEGRAL DDT-Zn·Cu-CITRIC ACID SAMPLES AFTER REACTION

Sample Exposure	Analysis*, %			
	DDEt	DDMS	DDD	DDT
336 hrs indoors	82.4	14.1	0.8	0.0
336 hrs out-of-doors	76.6	8.1	7.4	0.6
408 hrs indoors	84.0	12.9	0.6	0.2
408 hrs indoors (336 hrs, then add 1 g citric acid and 72 hrs additional)	83.9	13.0	1.0	0.0
408 hrs indoors (336 hrs, then add 1 ml acetic acid and 72 hrs additional)	84.9	13.1	0.5	0.0
Average	82.4	12.4	2.1	0.2

*Calculated as equivalent % DDT from which component was produced.

A similar series of tests was carried out in which sulfamic acid was employed as the acid source. These samples were prepared in the same manner as the citric acid samples, excepting that the 4.85 g (50 millimoles) of sulfamic acid was added in 50 ml of water (instead of the absolute ethanol used for citric acid), since sulfamic acid is insoluble in ethanol and similar organic solvents. There was a rapid bubbling and an odor of H_2S following acid addition. The mix was cooled in dry ice and stripped in a rotary evaporator; the operation was difficult because of the gas evolution. The hygroscopic mass was then ground and DDT added as before. The samples were than split into several portions, moistened and exposed as before.

Analyses of the samples after 0, 24 and 168 hr reaction indicated that 91.3, 74.5 and 88.0%, respectively, of the DDT remained in the three samples. It was then found that both the acid and most of the metal reductant had been consumed. It was first found that the extract from the 1 week sample was neutral, rather than pH 2-4 as known to be necessary for effective reaction. Hence, the evidence of little or no reaction appears due at least in part to the consumption of the acid. Some of the initial samples were treated with additional acid and tests were continued with no significant further reaction.

The experience with gas evolution from the reductant-acid mixture suggests that it would be desirable to obtain information on the extent of zinc consumption at various stages of the operation. Consequently, a device was set up to test evolution from the reductant residue by treating with hydrochloric acid and measuring the volume of hydrogen evolved. The data from several tests follows:

TABLE 20

ZINC CONSUMED ON ACID COATING AND AFTER REACTION WITH DDT

Sample	Unconsumed Zinc, %
Citric acid coated, before DDT application	82
Citric acid coated, after DDT application	57
Citric acid + DDT, 117 hr reaction	31
*Sulfamic acid + DDT, 168 hr reaction	19
*Sulfamic acid + DDT, 168 hr reaction	8
Sulfamic acid + DDT, 336 hr reaction	4

*H_2S odor.

These results show that substantially all of the zinc had been consumed in the sulfamic acid-coated samples tested, and that substantial reaction appeared to have taken place during the coating operation with the citric acid-treated samples.

IMPROVED CATALYST STUDIES

Studies were made of several systems that might give better catalysis than the copper couple employed to improve the reduction with zinc, aluminum, and iron. One hypothesis is that elements immediately to the left of the reductant on the periodic table should form p-type semiconductors that would provide a catalytic surface (Reference 6).

On this basis, the efficiency of cobalt and nickel in catalyzing zinc, aluminum, and iron was examined. Tests were carried out in which 1 meq of Cu^{++}, Co^{++}, or Ni^{++} was added to 1 g of powdered reductant to form the couple, 1 g of DDT was added in 20 ml of acetone, and the solution was made acid with 10 ml of 1.5 N acetic acid. Tests were run for 24 hr at 25°C unless otherwise indicated.

The results with zinc reductant follow in Table 21.

TABLE 21

EFFECT OF CATALYST ON ZINC REDUCTION OF DDT*

Component	Catalyst Couple:	Analysis**, %, Following 24 hr Reaction at 25°C Ni	 Co	 Cu***
DDEt		36.4	28.6	52.7
DBP		1.4	1.1	-
DBH		2.2	1.5	-
DDMU		4.7	5.0	-
DDMS		13.7	10.6	7.1
DDD		37.2	35.7	30.0
DDT		0	0	0
TTTB		3.1	8.9	0
DDA		1.7	1.2	0
	Balance	100.4	92.6	94.9

*Procedure employed was to dissolve 1 g DDT in acetone, then add 1 g of zinc dust followed by 1 meq of the designated catalyst ion to form the couple. The solution was made acid with 10 ml of 1.5 N acetic acid and stirred with a magnetic stirrer.

**Calculated as equivalent % DDT.

***A 2 hr reaction.

These results indicate that the copper couple formed in the same manner as nickel or cobalt is clearly superior in that the reaction was more rapid, and that the conversion to DDEt was more complete with less DDD as an intermediate product. A noticeable warming of the reaction flask was observed in the reaction with the Zn• Co couple.

Catalysis of aluminum by silver, nickel, cobalt, and zinc was also attempted. Again the results were inferior to those obtained when the copper catalyst was employed. The data are shown in Table 22.

TABLE 22

EFFECT OF CATALYST ON ALUMINUM REDUCTION OF DDT*

Component	Catalyst Couple:	Analysis**, %, Following 24 hr Reaction at 25°C				
		Ag***	Co	Ni	Zn	Cu
DDEt		5.0	0.6	0	0	0.6
DBP		6.7	0.3	-	0.6	6.9
DBH		-	-	-	-	4.8
DDMU		3.7	-	-	-	-
DDE		1.6	3.5	3.1	0.9	-
DDD		7.8	1.0	0.6	0.7	2.8
DDT		0.2	93.0	96.2	82.7	0.3
TTTB		33.3	0	-	0.2	36.6
DDA		10.0	0	-	-	38.0
Minor Components		-	-	-	-	1.1
	Balance	68.3	98.4	99.9	85.1	91.1

*Procedure same as Table 20 except aluminum powder reductant instead of zinc dust.

**Calculated as equivalent % DDT.

***72 hr reaction.

Attempted catalysis of iron reduction of DDT by cobalt or nickel couples was likewise inferior to the results obtained with copper. The results are given in Table 23.

TABLE 23

EFFECT OF CATALYST ON IRON REDUCTION OF DDT*

Component	Catalyst Couple:	Analysis**, %, Following 24 hr Reaction at 25°C		
		Co	Ni***	Cu
DDEt		-	0.9	0.6
DBP		-	-	1.1
DDE		2.7	4.1	1.7
DDD		1.8	3.8	8.2
DDT		96.1	73.2	8.5
TTTB		2.9	15.2	69.5
DDA		-	-	5.4
Minor Components		-	-	0.3
	Balance	103.5	97.2	95.3

*Procedure employed was to dissolve 1 g DDT in acetone, then add 1 g of CP powdered iron followed by 1 meq of catalyst ion solution to form the couple. The mixture was then made acid with 10 ml of 1.5 N acetic acid and stirred with a blade stirrer.

**Calculated as equivalent % DDT.

***25 hr reaction.

SECTION V

CHARACTERIZATION OF DDT DEGRADATION PRODUCTS

Important to the development of a system for the controlled degradation of field-applied DDT is an understanding of the physical and chemical properties of the degradation products. This enables one to determine whether the products are leached into runoff or ground waters, whether the materials are vaporized from the field, whether the products can be further reacted, are hydrolyzed by contact with water, etc. Accordingly, a limited program of characterization of the principal products from the degradation of DDT, DDEt and TTTB, was undertaken.

SOLUBILITY IN WATER

Solubility of DDEt in Water

The solubility of DDEt in water has been determined, essentially by the method of Biggar, Dutt, and Riggs (Reference 8). Purified p,p'-DDEt (25 mg) was dissolved in acetone and added to 1ℓ of water. The glassware used in these tests had not been exposed previously to DDT or degradation products and the water was deionized water that was additionally doubly distilled from glass. The acetone was removed by evaporating about three times the acetone volume from the water by heating on a steam-bath at 90-95°C for 20 minutes under a stream of nitrogen while stirring with a magnetic stirrer. The suspension was then cooled to ambient temperature, and the volume brought back to 1ℓ with water. It was then equilibrated with shaking in a 20°C constant temperature bath for 1 to 6 days. The samples were filtered through a fine (5μm) fritted glass filter, and the DDEt was extracted three times with pesticide-quality hexane. The combined extracts were evaporated to a known volume (0.5 - 1.0 ml) and analyzed by gas chromatography. The results of replicate tests are shown in Table 24.

TABLE 24

SOLUBILITY OF DDEt IN WATER AT 20°C

	ppb
	76
	65
	64
	63
	74
Mean	68
Standard deviation	6.6

This solubility is about 20-fold greater than the solubility of p, p'-DDT reported by Biggar, et al. (Reference 8) (3.4 ppb). The DDT solubility reported by Biggar is in substantial agreement with other determinations summarized by Gunther (Reference 9).

The same basic technique was used in attempting to determine the solubility of DDEt at 40°C. Purified p, p'-DDEt in acetone was added to doubly-distilled water, and the acetone was removed by evaporation. The samples were equilibrated with shaking in a 40°C water bath for 11 days.

Since the solubility of DDEt was expected to be somewhat greater at 40° than at ambient temperature, a method was required for removing the excess DDEt from the solution at the equilibrated temperature so that the temperature effect on solubility could be accurately determined. Accordingly, a filtering device was prepared for removing the undissolved DDEt from the DDEt-saturated water while maintaining the temperature of the solution at 40°. A filter stick (8 mm) with a fine (5 μm) fritted glass filter element was fused to glass tubing to form a U-shaped device that would filter directly from the thermostatted flask containing saturated DDEt (+ excess product) into a filter flask. Nearly all of the solution could be filtered in this manner while maintaining the solution at temperature. The unfiltered residue (~5 ml) was then measured and the solution volume corrected.

The DDEt was extracted three times with benzene, and the combined extracts were dried over anhydrous Na_2SO_4, concentrated to a known volume (1 ml), and analyzed by gas chromatography. However, complex gas chromatographic records were obtained instead of the expected single peak for DDEt. The presence of DBH was indicated in the samples (a few % of DDEt, exact amount difficult to establish) as well as DDEt. It appears that the higher temperature exposure of DDEt to water has resulted in hydrolysis of the compound.

Solubility of TTTB in Water

The solubility of TTTB was determined in a similar manner to the DDEt. A portion of purified TTTB (ca 10 mg) was placed in a flask and doubly-distilled water was added. Since TTTB was nearly insoluble in acetone, the material was added directly to water and it was shaken until equilibrium solubility was believed to be established. The samples were shaken in a gyratory shaking bath at 40°C for 9 days. The samples were filtered to remove undissolved TTTB by the method described for DDEt and the TTTB was extracted three times with benzene. The combined extracts were evaporated to dryness; no weighable residue was obtained (<0.1 mg). A more sensitive means of assay of the TTTB was required, since the compound does not respond to the gas chromatograph under the conditions employed.

It was found that the Schechter-Haller colorimetric method used for DDT, DDD, and DDE (Reference 10) could be employed for more sensitive assay of TTTB. This method consists of the nitration of the chlorophenyl groups, followed by development of a blue-green color by treatment with a benzene-methanolic sodium methylate solution. Maximum absorbence is at about 596 nm. The sensitivity of the method is about 1-2 μg or about 100-fold improved over the gravimetric method.

However, when the residues from the solubility tests were treated, no significant difference from blanks were obtained, so that the solubility of TTTB in water at 40°C appears to be < 1 ppb. Testing at 25°C was therefore not attempted.

SOLUBILITY IN FATS

An important characterization of the products of DDT degradation is the solubility in fatty tissues, since it is well known that DDT is concentrated through the food chain by virtue of its fat solubility. An indication of the solubility of the degradation products DDEt and TTTB in fats could be established from measurements with the liquid fat triolein (glyceryl trioleate). The solubility was established by incrementally adding either DDT, DDEt, or TTTB to filtered triolein, stirring until solubility was achieved, and adding further increments until the solution was saturated. The results of tests at ambient temperature (23-25°C) follow in Table 25.

TABLE 25

SOLUBILITY OF DDT, DDEt AND TTTB IN TRIOLEIN

Material	Solubility, %
DDT	13.0
DDEt	31.7
TTTB	0.27
TTTB*	0.39

*0.5% lecithin added to triolein.

These results show clearly that the degradation product TTTB is but slightly soluble in the fat, the solubility of the TTTB being 48-fold less than DDT in triolein. This result is in line with the early claims that TTTB would not have insecticidal properties because of limited fat solubility (Reference 11). DDEt, however, has somewhat greater solubility than DDT in triolein. This result is not unexpected because of the greater aliphatic nature of the DDEt molecule, compared to DDT.

SOLUBILITY OF TTTB IN VARIOUS SOLVENTS

While the product DDEt is freely soluble in a variety of organic solvents (acetone, benzene, hexane, etc.), little is known of the solubility of

TTTB in common solvents proposed for use in its preparation and purification. The results of a series of qualitative tests with a variety of solvents are presented in Table 26.

TABLE 26

SOLUBILITY OF TTTB IN VARIOUS SOLVENTS

Solvent	Solubility* of TTTB Ambient (23-25°C)	Hot
Methylene chloride		sl s
Chloroform	s	sl s
Carbon tetrachloride	sl s	
Benzene		sl s
Acetone		i
Methyl ethyl ketone	poor	s
Ethyl acetate		s
Ethanol		i
Isopropanol		i
Sec-butanol		sl s
Glacial acetic acid		sl s
Dioxan		sl s
Tetrahydrofuran (THF)		s
Chlorobenzene		s

*s = soluble, sl s = slightly soluble, i = insoluble

VAPOR PRESSURE

The vapor pressure is an important parameter in determining the persistence of degradation products under field conditions, since a moderately high vapor pressure may lead to volatilization of the products from the soil, particularly in warm areas. A survey of available techniques for determining the vapor pressure of pesticides revealed that the gas saturation method employed by Spencer, et al. (References 12, 13, 14) appeared best for the purposes of this study. The DDEt was placed on a silica sand support by dissolving 1 g in acetone, adding the acetone solution to washed sand (1 kg) and carefully evaporating the solvent. The DDEt-sand was then placed in a gas saturation tube (4 cm dia x 48 cm long), and nitrogen carrier gas was then saturated with the DDEt by slowly passing the gas through the saturator. The DDEt-laden gas was then passed through a gas washing bottle containing pesticide-grade hexane, which scrubbed the DDEt from the nitrogen. The hexane solution was then analyzed for DDEt by gas chromatography. The gas was freed of hexane by passing it through a solid CO_2-cooled trap and the gas volume was measured with a standard wet-test meter. Gas saturation was established from a constancy of measurement as the flow rate was changed. The results of several tests are shown in Table 27.

TABLE 27

VAPOR PRESSURE OF DDEt

N_2 Total Volume ℓ	Temp °C	N_2 Flow Rate ml/sec	Vapor Density of DDEt $\mu g/\ell\ N_2$	Apparent Vapor Pressure of DDEt mm Hg	Linear Velocity Through Saturator cm/sec
125	24	8.7	0.197	1.5×10^{-5}	0.70
125	24	4.5	0.144	1.2×10^{-5}	0.36
47	24	1.9	0.203	1.5×10^{-5}	0.15

In this treatment, the vapor pressure is calculated from the vapor density by the ideal gas equation.

These results show that the vapor pressure of DDEt is about 80-fold greater than p, p'-DDT (1.7×10^{-7} at 20°C, (Reference 15). The significance of this can be seen if one calculates the evaporation rate of DDEt as compared to DDT. The calculation of the evaporation rate of a series of compounds from glass plates has been made by Hartley (Reference 15) who found the rate of loss to be proportional to the vapor pressure multiplied by the square root of the molecular weight (diffusion process). A calculated comparison between DDEt and DDT evaporation rate is given in Table 28.

TABLE 28

CALCULATED EVAPORATION RATES OF DDEt AND DDT

Substance	Vapor Pressure mm Hg	M	Predicted Rate of Loss from Acre of Glass Plate
DDEt	1.4×10^{-5}	251	7.8×10^{-2} lb/day
DDT	1.7×10^{-7}	355	1.2×10^{-3} lb/day

On the basis of this calculation, DDT applied at the rate of 1 lb/acre would last a calculated 830 days. However, 1 lb of DDT degraded to DDEt (0.71 lbs) would last only 9 days. It would therefore appear that DDEt would be removed from the soil reasonably rapidly by evaporation. It should be noted that tests in which DDT in soil, or simulated field conditions, was degraded, the yield of DDEt, particularly at longer reaction times, has been lower than expected. Vaporization of the DDEt product is believed responsible for the apparent low yield observed.

STABILITY OF PRODUCTS TO HYDROLYSIS

The planned characterization of the products DDEt and TTTB includes the investigation of the fate of these materials under acid or basic hydrolysis conditions. These data are necessary in establishing what will happen to DDT degradation products after they are formed in the field.

In an initial experiment, 1.75 ℓ of doubly-distilled water was saturated with DDEt and filtered, and a 250 ml sample extracted to determine the DDEt content. The remaining sample was split into two 750 ml portions, one of which was acidified to pH 2.2 with sulfuric acid, the other was made basic with NaOH (pH 12.0). These samples were placed in a 20°C temperature bath (with shaking) and samples were withdrawn periodically. The samples were then extracted, concentrated, and analyzed. The gas chromatographs after 72 hrs showed no peaks other than DDEt at the initial concentration for both acidic and basic treatments. However, it should be noted that in the 40°C solubility measurement of DDEt, several % DBH was shown, apparently as a result of hydrolysis.

Similarly, TTTB (10 mg) was placed in water either made alkaline or acid (as above, pH 12.0 and 2.1, respectively), and equilibrated at 20°C. An extract of the samples revealed no products which would respond to the gas chromatograph after 72 hrs at 20°C.

REDUCTION OF TTTB

Since TTTB appears as a significant product when either catalyzed aluminum or catalyzed iron reductants are used for degrading DDT, it was of interest to determine whether this material could be further reduced by either the Zn• Cu or Al• Cu system. In these tests, 1 g of TTTB in 20 ml acetone was reacted with 1 g of the reductant, and the solution was acidified with 10 ml of 1.5 N acid. Acetic acid was used to acidify the Zn• Cu reaction and sulfuric acid was employed with the Al• Cu reductant. The reaction was carried out for 377 hrs (nearly 16 days) at ambient temperature. The gas chromatographic analyses showed no decomposition. Essentially complete recovery of unreacted TTTB was also shown.

IDENTIFICATION OF PRODUCTS

While the principal products, DDEt and TTTB, were identified earlier

(Reference 1), the identification and characterization of some of the other major products have also been attempted.

Identification of DDA

In tests with the Al• Cu reductant system using acetic acid to provide the requisite acidity, it was found that a substantial portion of the product mixture was not represented either by the TTTB precipitate or the products identified by gas chromatography analysis (DDEt, DDMS, DDD, DDT, etc.) of the acetone-soluble wash of the mix. Since this unknown represented a substantial product, its identification was attempted.

Samples from the Al• Cu-acetic acid reduction mixture after 24 and 168 hr reaction by the usual method were examined (Table 29). The residue from the acetone-soluble fraction was first compared with the analysis of components in this fraction by gas chromatography. The residue was substantially greater than the sum of GC-responsive components:

TABLE 29

RESIDUE AFTER EVAPORATION FROM
Al•Cu REDUCTION OF DDT

Reaction Time, hrs	Residue After Evaporation, %	Sum of Gas Chromatographic Responsive Components, %
24	72.3	4.9
168	44.2	10.7

The residue from the 24-hr reaction was then examined. It was found that a hexane extraction of the acetone-soluble residue dissolved 27.2% of the sample, leaving 41.5% insoluble matter. The hexane insoluble material was then dissolved in hot benzene and a product was removed by crystallization. The product had a melting point of 167°C. The material formed did not give a gas chromatograph response in 30 min.

A portion of the residue, crystallized from hot benzene, was then mixed with KBr and pressed into a cell for infrared analysis. The pellet was examined in a Perkin-Elmer Model 137B spectrophotometer. Absorbance was shown at 5.88, 6.74, 9.20, 9.89, 12.20, 12.40, 13.30, 13.55 and 13.80 μm. Tentative constituent group assignments were then made (Table 30).

TABLE 30

INFRARED SPECTRA OF PRODUCT FROM Al• Cu REDUCTION OF DDT

Wavelength, μm	Assignment
5.88	Carbonyl
6.74	p-Cl phenyl
13-14	Chlorine

A peculiar "smeared-out" absorbance was also shown in the 3-4 μm range. Upon examining the spectra tabulated by Burchfield and Johnson (Reference 16), the same absorbance was shown by DDA. When the spectra above were compared with authentic DDA, idential infrared spectra were obtained. A mixed melting point of the product obtained from the reaction with authentic DDA showed no depression of the melting point, demonstrating that the product obtained was DDA.

Preparation of DDNU

A suspected unidentified product of DDT degradation was the ethylenic analogue of DDEt, DDNU or 1, 1-bis (p-chlorophenyl) ethylene. This product was generally expected under the conditions which yield DDE. The material was prepared and its gas chromatographic response noted.

The material was prepared basically by the method of Grummitt (Reference 17). A sample of commercial grade di(p-chlorophenyl)methyl carbinol (Sherwin-Williams Dimite) was dehydrated to produce the desired compound. The material was heated at 210°C for 15 min and then was refluxed for 1 hr with 20% sulfuric acid. The crude product was washed with ice water, dissolved in ethanol, and decolorized with charcoal. The crystals had a melting point of 81.5-84.5°C. Upon recrystallization from isopropanol and decolorization with charcoal, colorless crystals with an 84-85°C melting point were obtained. The value compares well with the 84-86°C melting point reported by Grummitt (Reference 17), and 84-85.5°C reported by Garbisch (Reference 18).

Identification of 4, 4'-Dichlorobenzophenone (DBP)

A further product suspected on the basis of unidentified gas chromatography peaks was 4, 4'-dichlorobenzophenone. The identification confirmed its presence as a product (~1%) in the Al• Cu and Fe• Cu reduction of DDT.

This identification was confirmed by isolation of the material and measuring the melting point of the product and derivative. The dichlorobenzophenone was obtained (by crystallization from ethanol) from the residue remaining after removing the solvent from the acetone-soluble reaction products of an Al• Cu reduction of DDT. This material had a melting point of 143-145°C, compared to literature values ranging from 142 to 148°C, but mainly about 145°C (Reference 19). The 2,4-dinitrophenyl-hydrazone derivative was then prepared by the method of Haller, et al. (Reference 20), yielding orange crystals with a melting point of 237-239°C (Haller reported 238-240°C). It is therefore concluded that the product is 4,4'-dichlorobenzophenone.

PREPARATION OF MATERIALS

The quantities of DDEt and TTTB required for the fish toxicity testing (Section VI), as well as physical property characterization and chemical stability measurements required that these materials be prepared in pure form in moderate quantity.

Preparation of DDEt

In the initial attempt, the method of Becke and Buckschewski (Reference 21) was employed. In the attempt, chlorobenzene and paraldehyde were condensed in the presence of anhydrous $AlCl_3$ and dimethylformamide, with introduction of dry HCl. The product was fractionally distilled in vacuum. However, gas chromatography of a solution of the product showed that the distillate was a mixture of isomers and other impurities. Further attempts at distillation and crystallization did not yield a purified form of the p, p'-DDEt.

Better results appear to have been obtained from a forced reduction of DDT. In this preparation, 100 g of DDT was treated with 150 g of copper-catalyzed zinc over a period of 68 hrs; the reactant mixture was 0.5 N in sulfuric acid. Following the addition of the sulfuric acid, the temperature rose to 49°C, but gradually fell to ambient (∼ 25°C). The excess reductant was filtered off and washed with acetone. The acetone was removed by evaporation and the oily residue was extracted with benzene. Stripping of the benzene left 71 g of crude DDEt. Gas chromatography of this fraction showed it to be mainly DDEt with about 3% DDMS and DDMU, and 0.1% DDD and DDT. This material was then vacuum distilled with the major product distilling at 150-152°C at 1-1.2 mm pressure. The DDEt upon recrystallization from methanol yielded a product which was free from impurities as shown by the gas chromatograph. The melting point, 54-55°C (corrected) is the same as that reported by Grummitt, et al. (Reference 17).

Another batch of DDEt prepared by basically the same method showed the presence of small amounts of DDM, DDE, and DDMS by gas chromatography. Successive recrystallizations tended to diminish the amount of DDMS substantially, whereas contamination with DDE was reduced relatively little.

Further purification was required for the fish toxicity study, and removal of DDE was considered necessary. Column chromatography was therefore attempted in order to remove the DDE. Columns employing silica gel, alumina, Florisil (an absorbent consisting primarily of SiO_2 (84.0 ± 0.5%) and MgO (15.5 ± 0.5%); The Floridin Co.), and Sephadex (a partially alkylated, cross-linked dextran; Pharmacia Fine Chemicals, Inc.) were evaluated. The latter material, Sephadex, is a gel-filtration medium modified for use with polar organic solvents, and capable of separating components based on differences in molecular size.

One percent solutions of DDEt were employed as column feed. Hexane was used as the solvent with silica gel, alumina, and Florisil, and ethyl acetate with Sephadex. The ratio of absorbent to DDEt was 15:1 for all adsorbents except alumina, where a 7:1 ratio was used due to column dimensions. Treatment with the Sephadex resulted in complete removal of DDE, but had no effect on DDMS or DDM; furthermore, the eluate contained an additional impurity (of very short retention time), the nature of which is not known. Silica gel and Florisil both removed DDE, but neither removed DDD or DDMS. Alumina adsorbed DDE and DDMS, but had little effect on DDM. When the DDEt eluate from the alumina column was freed of solvent and the DDEt recrystallized once from methanol; the resulting DDEt was found to be 99.9% pure, the only contaminant being 0.13% of closely related DDM.

In an attempt to simplify the preparation process, a batch of DDEt was prepared by essentially the method used in previous preparations. That is, a 100 g lot of recrystallized DDT was reduced with 500 g of copper-catalyzed zinc to form the crude DDEt. The sample was reacted for about 48 hours (10 hours at reflux, the remainder at 23-25°C), and frequent samples were withdrawn for analysis. Analysis of the crude acetone solution at the end of reaction indicated 89.5% of the DDT had been converted to DDEt, 3.6% to DDMU, 3.0% to DDMS, and the remainder to minor products. Removal of the acetone, extraction with benzene, and recrystallization from cold methanol led finally to a crystalline white product with a melting point of 53-55°C. The gas chromatographic spectra indicated a DDEt purity of > 99% with the only impurity being DDMS. This procedure was used in preparing DDEt used in the fish toxicity testing.

Samples had been withdrawn from the batch during preparation in order to determine whether additional reduction with fresh zinc-copper couple, or Raney nickel, would remove the residual DDMU and DDMS found in the crude preparation. However, neither treatment was successful.

Further examination of test data suggested that the reduction of DDD rather than DDT might lead to a simpler preparation of pure DDEt with less DDMS impurity. In a test, 10 g of DDD in acetone was reacted with 50 g of zinc catalyzed by the slow addition of 50 meq of copper ion (i.e., normal ratio of 1 meq copper ion/g zinc). The reaction was made 0.5 N in sulfuric acid and was reacted at ambient temperature for 17 hr. After removal of the acetone, extraction with benzene, and recrystallization from cold methanol, a yield of 6.9 g of DDEt (theoretical yield 7.8 g) was obtained. The gas chromatographic analysis showed that the material was of better quality than the DDT-based preparation, with only a trace impurity (<<0.1%) of DDMS in the DDEt. Following this preparation, a 10-fold larger batch was prepared (100 g of DDD reacted). This batch gave an 80% yield of DDEt, with the only impurity shown by the gas chromatograph being $<0.2\%$ DDMS.

In another laboratory test, the reduction of DDA by Zn• Cu couple was attempted as a possible means for producing DDEt without DDMU or DDMS contamination. While some DDEt was produced (~12% DDEt, 5% DDM), the low yield suggests the procedure is impractical.

Preparation of TTTB

In view of the early experience of Bernimolin (Reference 22) and Riemschneider (Reference 11), where insecticidal effects at first attributed to TTTB were found to be associated with DDT impurities in the product, it was hoped that a preparation that did not involve DDT could be used. However, an examination of methods of preparation did not disclose a practical method of preparing this material that did not involve DDT. The method selected was the Al• Cu-H_2SO_4 reduction of DDT, known from previous data to give a high conversion to TTTB, followed by recrystallization until the sample would show no trace of DDT upon gas chromatographic analysis.

A 5 g sample of DDT was reacted in 100 ml of acetone with 5 g Al• Cu couple to which 50 ml of 1.5 N H_2SO_4 was added to provide the requisite acidity. The reaction was then carried out for 48 hrs at ambient temperature. The temperature rose from 21°C to 30.5°C (about 3 min after the inception of reaction), but returned to nearly ambient conditions within 40 minutes. After the 48-hr reaction period, the crude TTTB and unreacted Al• Cu were separated from the remainder of the reactants by centrifugation. The crude TTTB (+ Al• Cu) was extracted four times with acetone, which removed most of the DDT. The TTTB was then extracted with hot $CHCl_3$ and the solvent removed, leaving 3.80 g (76% yield) of TTTB. The partially purified TTTB was recrystallized from tetrahydrofuran, and placed in a micro Soxhlet extractor and extracted with boiling acetone. Three extractions with acetone were made, and a decrease in impurities was noted in the gas chromatographic trace after each extraction. Final purification was achieved by recrystallization from ethyl acetate. A portion of the final product was dissolved in benzene and analyzed by the gas chromatograph; no residual DDT, DDD, nor other degradation products of DDT were found. Based on the quantities employed, the level of DDT, DDD, or similar impurities must be less than 0.001% in the product. The melting point of the product was 268-270°C. A scale-up of this basic method was used for the preparation of larger batches of TTTB employed in fish toxicity testing.

SECTION VI

TOXICITY TESTING OF DDT-DEGRADATION PRODUCTS

The second phase of the program involved studies designed to assess the toxicity of DDEt and TTTB to selected aquatic organisms. One of the objectives was to determine, by means of acute bioassay tests, the 96-hr median tolerance limit (TLm) of both the above products to three fish species and to one aquatic invertebrate which would be representative of a fish food organism. The second objective was to determine, by means of long-term continuous exposure studies, the maximum acceptable DDEt and TTTB concentrations for the fathead minnow. The maximum acceptable concentration would be estimated on the basis of data collected on growth, survival, and reproductive capacity of the test organism. A third objective was to utilize the data collected from both types of tests on the fathead minnow for the calculation of application factors for both products and to utilize these application factors for predicting the maximum acceptable DDEt and TTTB concentrations for the other two species of fish involved in the study. The fathead minnow was selected as the basic test organism because of its wide use as a reference test fish in toxicity testing.

The method used for predicting safe toxicant concentrations was proposed by Mount and Stephans (Reference 24). The method requires an estimate of the 96-hour TLm and the maximum acceptable toxicant concentration (MATC) of a given toxicant for a reference fish species (in this case the fathead minnow). The application factor is calculated by dividing the MATC by the value for the 96-hr TLm. The MATC of the same toxicant for a second fish species can then be estimated by multiplying the 96-hr TLm concentration of the toxicant for the second fish species by the application factor.

EXPERIMENTAL CONDITIONS

Test Animals

The three species of fish selected for the study were the fathead minnow (*Pimephales promelas*, Raphinesque), the bluegill (*Lepomis machrochirus*, Raphinesque) and the rainbow trout (*Salmo gairdnerii*, Richardson). Fathead minnows of mixed ages were purchased from Jim's Sportshop in Gilroy, California, and shipped by air carrier to the El Monte laboratory. Two purchases of approximately 800 fish each were made, the first on January 20, 1971, and the second on May 18, 1971. The fish purchased were sexually immature and measured approximately 2 in. in length.

The minnows were acclimated in the laboratory for at least two weeks before being used in any test. During the acclimation period, they were kept in round polyethylene tanks containing about 20 gallons of water which was changed continuously and held at $23 \pm 1^{\circ}$C. During the first week after acquisition, the minnows were treated with tetracycline-HCl

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(Richlyn Labs, Inc., Philadelphia) to minimize bacterial infections. Tetracycline was administered at a rate of 250 mg per five gallons of water. The water was kept static during treatment. All fish were fed Oregon Moist Trout Pellets (R. V. Moore, Inc., LaConner, Washington), ad libitum, once a day except on weekends.

The bluegill and rainbow trout were purchased from Corcoran Brothers of Azusa, California, and brought to the laboratory by truck. The bluegills were purchased on 27 October 1971, and the trout on 10 November 1971. Both fish species were acclimated in the laboratory for at least two weeks before being used in any test. During the acclimation period, the fish were kept in 15-gal aquaria containing about 10 gal of water. The water was changed continuously and maintained to within ± 1 degree at 12°C and 23°C for the trout and the bluegill, respectively. These fish were not treated with antibiotic. The bluegills were fed ad libitum once daily on Oregon Moist Trout Pellets and the trout were fed Purina Trout Chow in a similar manner.

Daphnia magna were obtained from the National Water Quality Laboratory in Duluth, Minnesota, in January 1971, and propagated in the Envirogenics Systems Company Aquatic Biology Laboratory. These microcrustaceans were kept in 15-gal aquaria and fed compressed yeast supplemented by phyto- and zooplankton cultured in an infusion made from filtered pond water and horse manure.

Laboratory Water Supply

The source of the water used in the laboratory was the El Monte Municipal water supply, obtained from local wells. Water entering the laboratory was first passed through activated charcoal columns (Sparkletts Water Co.) and then through an electronic liquid sterilizer (Aquafine Corp., Model SL-1, Los Angeles). The sterilizer is an ultraviolet light equipped with a 260 nm wavelength monitor and alarm system. The purpose of the sterilizer was two-fold. One was to convert any residual chlorine present as hypochlorite to gaseous Cl_2 for later air stripping. The second objective was to reduce the number of microorganisms that might have entered with the water.

A portion of the treated water was piped through a stainless steel heat exchanger operating off of a 250-ton plant refrigeration system. This system was designed to provide chilled water to the test laboratory. Water leaving the heat exchanger, as well as the bypass water from the sterilizer, was piped into head tanks located within each test laboratory.

The heat tanks, constructed of acrylic plastic (Plexiglas), were equipped with stainless steel temperature-controlled heating units. The heating units were used only when necessary. Each head tank was also equipped with a spinning-disc aerator. The disc aerator consisted of about 75 mylar discs measuring 4 in. in diameter, mounted 1/4 in. apart on a stainless steel rod by means of polyethylene spacers. The device was mounted horizontally across the head tank so that one-half of each disc was exposed to air. The shaft was then rotated by an electric motor equipped with a speed control. The device provided water saturated with oxygen to the diluters.

Each head tank was designed to deliver water to a Mount-Brungs proportional chemical diluter (Reference 23) and to a manifold constructed of polyvinyl chloride plastic tubing. The manifold permitted delivery of water to the individual test tanks when necessary.

The results of a chemical analysis of the water are shown in Table 31.

ACUTE TOXICITY STUDIES

Acute Toxicity Studies on Selected Fish Species

Experimental Design

These studies were designed to provide data for estimating the 96-hr median tolerance limit (TLm) of DDEt and TTTB for the fathead minnow, bluegill, and rainbow trout. The bioassay procedures used in these studies were those of Standard Methods (Reference 25), modified slightly.

Acute bioassay tests, designed to determine the 96-hr TLm concentration of DDEt for fathead minnows, were conducted in static test solutions, and the effects of aeration and DDEt-dispersing agents were evaluated. Initial concentrations of DDEt as high as 100 ppm were used. Acute tests involving TTTB and the fathead minnow were carried out only with TTTB-saturated water; dispersing agents to increase the amount of suspended TTTB were not used. All test fish were fasted for 24 hr prior to testing and food was withheld throughout the duration of each test.

The acute tests with bluegill and rainbow trout involved the use of a flow-through toxicant delivery system designed to achieve continuous renewal of the test medium. The tests were conducted in 1 x 1 x 2 ft (HWL) glass aquaria outfitted with drains which allowed the tanks to fill to a depth of 10 in. At this depth each tank held about 50 liters of test solution.

Laboratory water was delivered to each test container at a rate of 200 ml per min, providing a turnover of approximately 5.75 tank volumes per day. Water temperatures were maintained at $12 \pm 1^{\circ}C$ and $23 \pm 1^{\circ}C$ for the trout and the bluegills, respectively.

Acute toxicity testing with the bluegill and trout were conducted at one concentration only -- the saturation level of DDEt or TTTB in water, since earlier tests with the fathead minnows indicated that the toxic limit would probably be no lower than saturation. These values were, respectively, ~ 0.05 and ≤ 0.001 ppm.

Each toxicant was dissolved in acetone before being mixed with the stream of water being delivered to the test container. Delivery of the acetone solution was accomplished by using a Sage syringe pump

TABLE 31

CHEMICAL ANALYSIS OF RAW WATER SOURCE FOR FISH BIOLOGY LABORATORY

	mg/ℓ		mg/ℓ
Total Hardness (as $CaCO_3$)	148	Phenol	<0.001
Calcium	40.6	Chlorides	13.6
Magnesium	11.2	Fluorides	0.96
Potassium	0.5	Cyanides	<0.0005
Sulfates	7.8	Iron	<0.001
Sulfides	<0.002	Copper	<0.005
Nitrates	<0.65	Zinc	<0.001
Nitrites	0.005	Cadmium	<0.010
Ammonia	0.45	Chromium	0.025
		Lead	<0.040

Total Alkalinity	161 mg/ℓ $CaCO_3$
pH	7.65 at 25°C
Specific Conductance	389 μmhos/cm
Chloramines	0.004 mg/ℓ

(Model 255-1) fitted with a 50 cc syringe. The concentration of DDEt in the test tank was determined by analyzing samples of the test solution by gas chromatography, after hexane extraction and concentration in a Kuderna-Danish evaporator. The concentration of the saturated TTTB solution was estimated on the basis of water delivery rate, the concentration of the TTTB-acetone solution, and information previously obtained on the solubility of TTTB in water; the concentration of TTTB in water was too low to measure accurately by any available means.

The survival of each fish species in DDEt- or TTTB-saturated water was compared with the survival of the same species in unadulterated laboratory water. Each test was conducted once, and the duration of each test was 96 hrs. Food was withheld for 24 hr prior to testing and during the tests. The number of trout and bluegill tested per tank were 5 and 10, respectively. The mean weights and lengths of the test fish are listed in Table 32.

TABLE 32

MEAN WEIGHT AND LENGTH OF TEST BLUEGILL AND TROUT USED IN ACUTE TOXICITY TESTING

Toxicant/Fish Species	Controls		Toxicant Exposed	
	Weight (g)	Length (mm)	Weight (g)	Length (mm)
DDEt/bluegill	1.73	49.6	1.66	48.5
TTTB/bluegill	1.94	51.8	1.75	50.6
DDEt/trout	31.5	154	31.3	156
TTTB/trout	34.2	152	34.5	152

Data and Discussion

Test mixtures, in which up to 100 ppm DDEt were added, were prepared by mixing measured amounts of DDEt in acetone with two liters of water. These mixtures had no toxic effect on fathead minnows exposed for 96 hours. Analysis of filtered samples of the test mixtures showed DDEt to be present in solution at concentrations no higher than 0.05 ppm. The remaining DDEt added was precipitated. It was observed that mixtures, made to contain DDEt in concentrations exceeding 0.05 ppm, contained relatively large particles of precipitated DDEt on the surface of the water. A fresh set of the same mixtures were prepared and gently aerated to keep the undissolved particles more evenly distributed. As in the previous test, 2 minnows were placed in each test container (only 2 fish were used since this was a screening test). The test results were the same as with the unaerated mixtures; no toxic effects were noted on the fish.

These tests indicated that the 96-hr TLm concentration was greater than 0.05 ppm (or saturation) and that a method of preparing stable suspensions of DDEt was needed.

Exploratory tests with two emulsifiers were conducted. An acetone solution of DDEt was mixed with Triton X-100, a dispersing agent recommended by Mount and Brungs (Reference 23), to form a suspension containing 1.56 ppm DDEt and 1.88 ppm Triton X-100. The suspension was allowed to stand undisturbed for 48 hr. During this time, most of the DDEt had settled and samples taken above the residue contained no more than 0.05 ppm DDEt. Fish exposed to this suspension for 96 hr survived well.

Further tests were conducted using an agent commonly employed in preparing emulsifiable concentrates of DDT. This agent was Agrimul (Napco Chemical Company), a non-ionic ether-linked dispersant. This dispersant was evaluated both with and without added aromatic solvent. In the first test, 6.3 mg of Agrimul and 1.0 ml of acetone containing 15 mg of DDEt were mixed; then 100 ml of water was added and the mixture shaken to disperse the DDEt. The emulsion was then added to 2525 ml of water, mixed and allowed to stand. A second mixture was prepared in a similar manner except that 1.0 ml of xylene was added (xylene is commonly used in field-applied formulations). After both mixtures had stood for 24 hr, they were still quite cloudy, indicating that both contained a substantial amount of DDEt in suspension. A 1.0 ℓ sample was withdrawn from each mixture, extracted 3 times with hexane, concentrated to 1.0 ml and analyzed by gas chromatography.

In the preparation without xylene, 2.6 mg/ℓ, or 46% of the initially added DDEt remained in suspension. In the preparation containing xylene, 5.1 mg/ℓ, or 89% of the initially added DDEt remained in suspension. These experiments demonstrated that Agrimul 70A aided substantially in keeping DDEt in suspension and that xylene strikingly augmented this effect.

Bioassays on these mixtures followed. The xylene mixture, containing 2.2 ppm Agrimul and 310 ppm xylene killed all of the test fish within 30 sec. On the other hand, the mixture without xylene, and with the same Agrimul content, did not affect any of the test fish during a 6-day exposure period. The toxic effect observed in the first test was deemed due to the xylene.

Accordingly, fish were then exposed to an aqueous suspension containing 2.2 ppm Agrimul and 10 ppm DDEt for 6 days. At the end of the exposure period, all of the fish were alive and showed no untoward behavior. Analysis of the suspension after 23 hours showed that 6 ppm DDEt (60%) remained in suspension at the conclusion of the test. Another bioassay was conducted on a suspension initially containing 2.2 ppm Agrimul and 100 ppm DDEt. The mixture was very milky in appearance. A single fish placed in this suspension was alive after 6 days of exposure. However, after 72 hr, the fish lost and did not regain its equilibrium. Analysis of the suspension after 71 hr showed that 16.1 ppm DDEt remained in suspension. It appeared that most of the DDEt was precipitated early in the test so that the time-average concentration was probably near 16. 1 ppm.

Further attempts to determine the 96-hr TLm of DDEt for the fathead minnow were abandoned. Tests conducted demonstrated that exposure of the minnows to a saturated solution of DDEt (50 ppb) for 96 hr was not harmful. Addition of Triton X-100 or Agrimul 70-A helped to keep DDEt in suspension, but the amount maintained in suspension was neither consistent nor long-lasting. Although the fish exposed to a suspension of Agrimul and 16 to 100 ppm DDEt were affected, the question remained as to whether loss of equilibrium was due to DDEt alone or the extreme turbidity of the suspension.

Attempts to determine the 96-hr TLm of TTTB for fathead minnows were not made; tests were run only at saturation where no effect was noted. Subsequent data from acute tests on TTTB with bluegills and rainbow trout, as well as results obtained from the chronic studies on TTTB with fathead minnows, suggest that if minnows were exposed to a saturated solution of TTTB ($\leqslant$1 ppb), for 96 hr, they would not be adversely affected.

The data in Table 33 indicate that, like the fathead minnow, the bluegill and rainbow trout can survive well in DDEt- and TTTB-saturated water for 96 hr. No toxic symptoms were observed.

TABLE 33

MORTALITY AFTER 96 HOURS EXPOSURE

Toxicant (Conc.)	Fish Species	Mortality/No. Exposed	
		Control	Toxicant Exposed
DDEt (50 ppb)	Bluegill	0/10	0/10
TTTB ($\leqslant$1 ppb)	Bluegill	0/10	0/10
DDEt (50 ppb)	Rainbow trout	0/5	0/5
TTTB ($\leqslant$1 ppb)	Rainbow trout	0/5	0/5

The reason for limiting the maximum test concentrations of DDEt and TTTB to 0. 05 and $\leqslant$0. 001 ppm, respectively, in the tests utilizing the bluegill and the rainbow trout was two-fold. First, earlier attempts at preparing uniform suspensions of DDEt containing greater than saturated concentrations met with failure. TTTB is only slightly soluble in acetone, which is considered a reasonably non-toxic organic solvent having a 96-hr TLm of about 10, 700 ppm (Reference 24). To attempt to develop a method for satisfactorily maintaining both compounds in suspension would have been time-consuming. Secondly, toxicity data from tests involving the use of solvents and dispersants in combination with the test compound are often meaningless. Often, the observed toxic effects are due almost entirely to the solvent or emulsifier

(Reference 26). Workers at the Duluth EPA laboratory recommended against attempting to run acute toxicity tests at concentrations greater than the solubility of the toxicant in water.

DDEt is no more than and apparently much less than half as acutely toxic to fathead minnows, bluegills, and rainbow trout as DDT. According to Henderson and co-workers (Reference 27), the 96-hr TLm concentration of the 100% p, p'isomer of DDT for fathead minnows in soft and hard water is 0.026 ppm. Tests on the technical grade of DDT gave 96-hr TLm estimates of 0.021 ppm for bluegills, 0.042 ppm for fatheads, 0.036 ppm for goldfish, and 0.056 ppm for guppies. The 96-hr TLm concentration of an unspecified grade of DDT for rainbow trout is reported in Water Quality Criteria (Reference 28) as ranging from 0.024 to 0.074 ppm. Since the solubility of TTTB in water is only about 0.001 ppm or less, no comparison can be made with this toxicant.

Since TLm estimates on DDEt and TTTB for the three species of test fish could not be obtained, the third objective, that of calculating the maximum acceptable DDEt and TTTB concentrations for rainbow trout and bluegills, could not be stated. Indeed, the TLm of DDEt for the fathead minnow, bluegill, and rainbow trout can only be stated as >0.05 ppm, and for TTTB, $>.001$ ppm.

Acute Toxicity Studies on Daphnia Magna

Experimental Design

These tests were designed to provide data for estimating the 96-hr TLm of DDEt and TTTB for the microcrustacean, Daphnia magna. For comparative purposes, similar tests were conducted on DDT.

The maximum concentration of each compound tested was that obtainable at saturation at room temperature, which ranged from 22-24°C. Saturated solutions were prepared by shaking an excess amount of the compounds in 2 liters of laboratory water for 48 hr and filtering off the undissolved material. Gas chromatographic analysis of samples showed 46-55 ppb DDEt and 0.9 ppb DDT, respectively. The TTTB concentration from solubility tests was estimated to be 1 ppb or less.

The first series of tests was carried out on DDEt and DDT. Ten daphnids, less than 24 hr old, were placed in 400 ml beakers containing 200 ml of unaerated water with different amounts of DDEt or DDT. Every 24 hr, for 96 hr, the dead daphnids were removed and counted. Since it was occasionally difficult to determine if a carcass was actually a dead daphnid or a cast, the survivors were also counted. Food was withheld during the tests. The tests were repeated once. The data are shown in Tables 34 and 35.

TABLE 34

CUMULATIVE PERCENT MORTALITY OF D. MAGNA EXPOSED TO DIFFERENT CONCENTRATIONS OF DDEt AND DDT (Test 1)

	Mortality % after Test, hr								
	DDEt					DDT			
Conc. (ppb)	24	48	72	96	Conc. (ppb)	24	48	72	96
0	0	0	50	50	0	0	10	40	60
0	0	0	0	40	0	0	0	20	20
0.09	10	20	30	50	0.0009	0	30	30	40
0.9	10	20	50	60	0.04	0	0	30	40
9.2	10	10	10	20	0.09	0	10	20	40
23.0	0	20	40	50	0.45	0	40	100	100
46.0	0	30	50	60	0.90	0	80	100	100

TABLE 35

CUMULATIVE PERCENT MORTALITY OF D. MAGNA EXPOSED TO DIFFERENT CONCENTRATIONS OF DDEt AND DDT (Test 2)

	Mortality % after Test, hr								
	DDEt					DDT			
Conc. (ppb)	24	48	72	96	Conc. (ppb)	24	48	72	96
0	0	0	0	30	0	0	10	50	100
0.09	0	0	0	0	0.009	0	10	10	70
0.9	0	10	10	20	0.04	0	10	30	50
9.2	0	10	10	10	0.09	0	30	30	50
23.0	0	20	30	30	0.45	0	0	10	10
46.0	0	0	0	0	0.90	10	60	70	80

A second series of tests, utilizing the same test concentrations, was carried out subsequently. In these tests, the test solutions were renewed every 24 hr and 1 ml of an 0.2% yeast suspension added as food. The data are shown in Table 36.

TABLE 36

CUMULATIVE PERCENT MORTALITY OF D. *MAGNA* EXPOSED TO DIFFERENT CONCENTRATIONS OF DDEt AND DDT AND FED YEAST*

	After Test, hr								
	DDEt					DDT			
Conc. (ppb)	24	48	72	96	Conc. (ppb)	24	48	72	96
0	0	0	20	20	0	10	10	10	10
0.092	0	0	0	0	0.009	0	0	30	30
0.92	0	0	0	0	0.045	10	20	30	40
9.2	0	10	10	20	0.09	0	0	0	0
23.0	0	20	30	30	0.45	20	20	20	30
46.0	10	10	10	10	0.90	0	0	0	10

*1.0 ml 0.2% yeast suspension per day, flow-through system.

Tests were also conducted on various dilutions of DDEt- and TTTB-saturated water with mature daphnids as test animals. Test conditions were the same as those used in the first test series; however, the length of the tests was shortened to 48 hr; no census was taken until the 48th hr and 20 animals were exposed to each concentration.

Data and Discussion

Mortality of the yound daphnids exposed to DDT or DDEt was inconsistent (Tables 34, 35, and 36). Mortality among controls was unusually high, and in some cases even greater than that of the daphnids exposed to the highest toxicant concentrations. The addition of food and renewal of the test solutions lowered mortalities in most cases, but did not improve the consistency of the mortality rate.

Tests on mature daphnids provided considerably more consistent data (Table 37). For DDEt the dose-response curve (Figure 1) was essentially linear between 13.2 and 55 ppb. The 48-hr TLm was estimated at 35 ppb. The toxicity of DDEt and DDT to *Daphnia* appears similar. Anderson (Reference 29) reported that *D. magna*, exposed to DDT concentrations ranging from 1 to 100 ppb, were immobilized in periods ranging from 16 to 32 hrs. Frear and Boyd (Reference 30) estimated the 26-hr TLm concentration of DDT for *D. magna* as 4.4 ppb. For *D. pulex*, the 48-hr TLm concentration of DDT was estimated at 36 ppb (Water Quality Criteria, Reference 31).

TABLE 37

PERCENT MORTALITY OF ADULT D. MAGNA EXPOSED TO DDEt OR TTTB FOR 48 HOURS

DDEt		TTTB	
Conc. (ppb)	% Mortality	Conc. (ppb)	% Mortality
0.00	5	0.00	10
2.75	0	0.05	0
7.43	0	0.14	0
13.2	10	0.24	0
23.05	25	0.42	5
41.2	60	0.75	5
55.0	90	1.0	0

CHRONIC TOXICITY STUDIES

Chronic Study with DDEt-Saturated Water

This study was originally started with four DDEt concentrations in addition to control. Except for control, the least concentrated test solution contained approximately 0.05 ppm DDEt which is approximately the solubility limit of DDEt in water at 20-25°C. After the tests were underway, problems in maintaining DDEt in suspension at levels exceeding saturation made it necessary to abandon tests on concentrations exceeding 0.05 ppm.

The study on the long-term effects of DDEt-saturated water on growth, survival and reproduction of the fathead minnow was continued. During the investigation which was initiated on March 24, 1971, the parental fish were exposed continuously to DDEt-saturated water for a period of 28 weeks. A description of the equipment and procedures employed follow.

The experiments and equipment were designed according to recommendations provided by the EPA Laboratory in Duluth, Minnesota. The testing facilities permitted continuous renewal of the test solutions in the test containers. Both control and DDEt-exposure tests were conducted in duplicate.

The test tanks were placed on a double-shelved metal rack with the larval tanks above the adult tanks, and were illuminated by two-tube sets of 4-ft Durotest "Optima" fluorescent lamps set 18 in. above the tanks. A plastic mixing cell, 4 x 2 x 6 in. high, was positioned above each pair of larval tanks to receive the treated tapwater used in the tests. Each mixing cell contained two outlets designed to distribute the

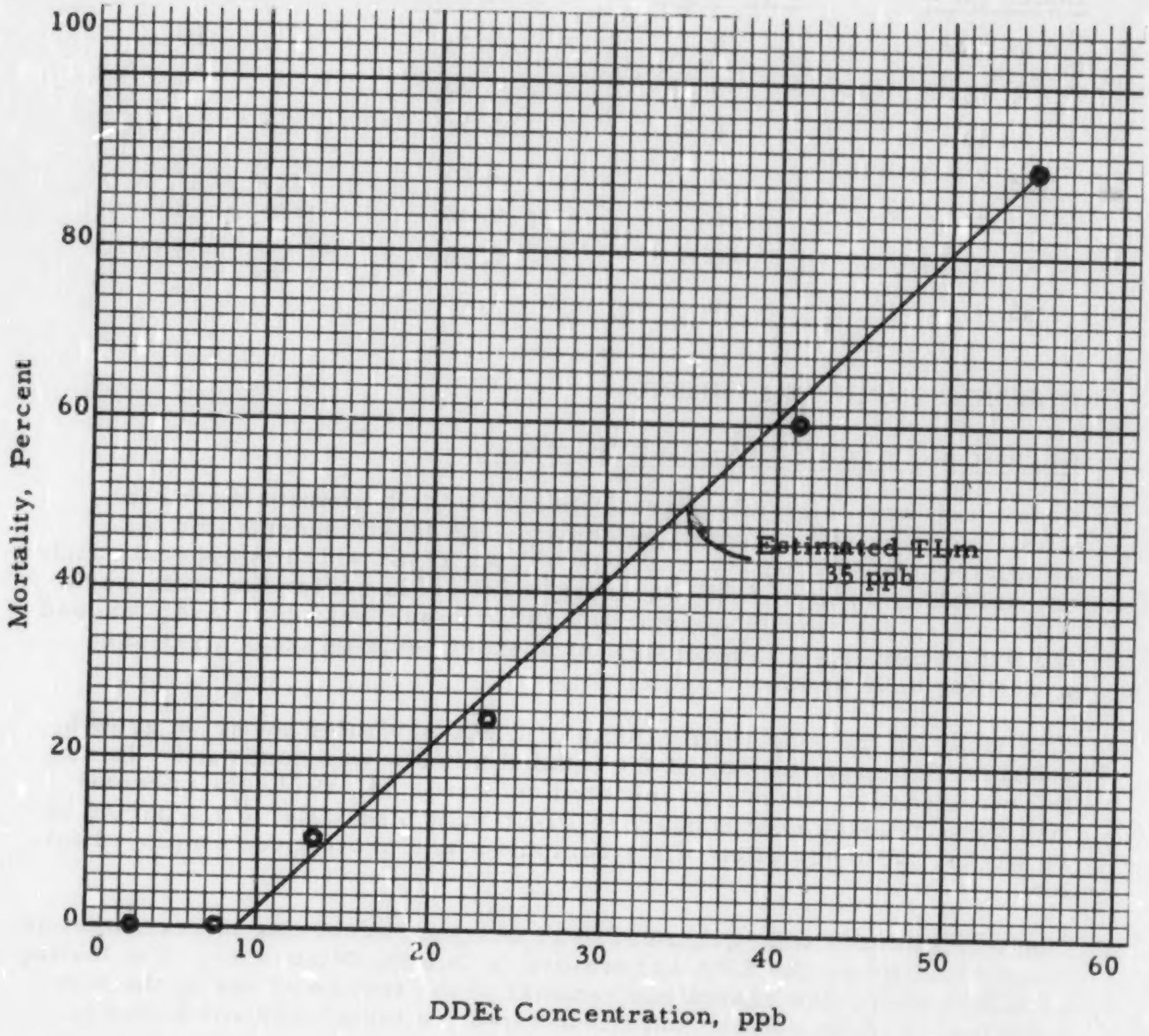


Figure 1. Dose-Response Curve for Daphnia magna exposed to Solutions of DDEt for 48 hours

water evenly between each of the duplicate larval tanks. Water flowed from the larval tanks into the lower adult tanks and then into a drain.

The depth of the water in all of the tanks was 6 in. At this depth, the larval tanks held about 14 ℓ of water and the adult tanks twice as much. The water delivery rate to each larval-adult tank set was 200 ml per min. The turnover rate for the larval and the adult tanks was about 10 and 5 tank volumes per day, respectively.

DDEt-saturated water was prepared by metering an acetone solution of DDEt, containing a known amount of the compound, at 0.01 ml per min, into one of the mixing cells with a Sage syringe pump (Model 255-1). Complete mixing of the toxicant with the incoming water was accomplished by employing a magnetic stirrer. Analysis of the DDEt solution showed an average DDEt concentration of 0.05 ppm.

Fifteen sexually immature minnows, selected from the stock procured on January 20, 1971, were weighed and measured for length and placed in each adult tank. They were weighed and measured again at the end of the study. Oregon Moist Trout Pellets were provided ad libitum once daily except on weekends. Water temperature was maintained at 23 ± 1°C throughout the test. The fluorescent lamps, controlled by an automatic timer, were turned on at 6:00 a.m. each day and were turned off according to the photoperiod schedule included in the appendix. Initial day length was set for 13 hr, corresponding to April 1 on the schedule. Day length was adjusted on the 1st and 15th of each month. Except on weekends, daily observations on behavior were made and mortalities recorded.

When the males began to display sexual colors, three spawning tiles, constructed of 4-in. half-sections of 6-in. diameter asbestos-concrete drain pipe, were placed in each adult tank. The tiles were inspected for eggs after 12 noon each day except on weekends. Eggs from each nest were removed from the tiles and transferred to petri dishes for counting. Since several females often deposit eggs in the same nest, the eggs were examined for stage of development through a low-powered binocular microscope. Eggs in distinctly different developmental stages were counted as separate spawns.

Usually 50 unbroken eggs were selected from each non-weekend spawn and incubated by the rocking egg cup method described by Mount (Reference 32). The egg cups were constructed of 3-in. sections of 2-in. diameter, heavy walled polyethylene pipe with Nitex screening material (40 meshes per in.) heat-sealed to one end. An electric motor was used to gently oscillate the cups containing the eggs up and down in the test medium 3 times per min. The eggs were inspected daily and the dead eggs removed. When the eggs began to hatch, they were left undisturbed until hatching was complete.

The larvae were counted and 25 or more transferred to rearing chambers and reared for 30 days. The larvae were fed a slurry of Oregon Moist Trout pellets mashed finely in water ad libitum once a day, except on weekends. The surviving larvae were counted and the caudal length recorded at the end of the 30-day test.

Data and Discussion

During the 28-week exposure period, the adult fish showed no signs of distress. Very few adult fish died during the test. Percent survival for controls was 97% or 29 of 30 fish. Percent survival for DDEt-treated fish was 87% or 26 of 30 fish. Fish that died did not show symptoms of the so-called "benzene bends" which have been observed in minnows known to have died from DDEt-exposure (see later in this section). There are insufficient data to indicate whether there is a statistical difference in survival rate of the control group and the DDEt-treated colony.

To evaluate the effects of DDEt on the growth of the adults, all fish were weighed and measured for length at the beginning and at the end of the study. The initial mean length, calculated by averaging the initial lengths of fish in both treatment groups, was 55.2 mm. This mean was subtracted from the individual lengths measured at the end of the test. The difference was considered the individual change in length. The mean gain in length was 9.5 mm for controls and 8.0 mm for the DDEt-treated fish. The difference in the mean gains was not significant by the Students t-test ($t_{calc} = 1.095$; $t_{.05} = 2.01$). There was, however, an appreciable difference in the gain in weight. The initial common mean was 2.30 g. The mean gain for controls was 1.46 grams and for the DDEt-treated fish, 0.92 g ($t_{calc} = 7.13$; $t_{.05} = 1.81$).

Spawning commenced on 13 May and continued until 5 August. The number of spawns produced by each duplicate colony varied, but the pooled totals were similar (Table 38). The controls spawned 23 times while the DDEt-treated fish spawned 24 times. Exposure to DDEt also did not appreciably affect the number of spawns produced per female. The average spawns per female for controls was 1.38; for the DDEt-treated colony, the average was 1.32. Although the DDEt-treated colonies produced 140% more eggs than the control colonies, the variation in the number of eggs produced per spawn was so great that there was no significant difference in the mean number of eggs produced per spawn ($t_{calc} = 0.51$; $t_{.05} = 1.68$). Exposure to DDEt also had no effect upon egg hatchability.

Data from 30-day growth and survival tests, carried out on larvae hatched from eggs incubated under control conditions and in DDEt-saturated water, indicated a deleterious effect of DDEt. Of 6 groups of larvae reared in DDEt-saturated water, 4 suffered total mortality within two weeks. Less than half of the initial number of larvae in the remaining two groups survived the 30-day test. Survival of larvae in seven control groups ranged from 38% to 98%. The average was 67%. Growth, as measured by peduncle length, was not affected. The data are presented in Table 39.

TABLE 38

REPRODUCTION DATA FOR FATHEAD MINNOWS EXPOSED TO DDEt-LADEN WATER

	Control		DDEt-Treated Fish	
Group:	A	B	A	B
No. of males	6	7	5	7
No. of females	9	8	10	8
No. of spawns	9	14	14	10
Spawns/female	1.0	1.8	1.4	1.2
Av. eggs/spawn	171 ± 60	228 ± 342	359 ± 354	157 ± 91
Total eggs produced	1540	3189	5029	1573
Percent hatch(N)	74 (3)	83 (11)	74 (9)	75 (5)

TABLE 39

THIRTY-DAY LARVAL GROWTH AND SURVIVAL OF FATHEAD MINNOWS IN CONTROL AND DDEt-SATURATED WATER

	Control	DDEt-treated
No. of test groups	7	6
Mean survival (%)	67 ± 23	15 ± 23
Mean length	8.9 mm	8.6 mm

Six additional larval growth and survival tests were conducted on larvae which had hatched from control eggs and were then exposed to water saturated with DDEt. None of the larvae survived longer than 10 days. The test data indicate that continuous exposure to DDEt-saturated water, containing about 0.05 ppm DDEt, does not affect behavior or survival of adult fathead minnows. Although exposure to DDEt has no effect upon growth as measured by increase in length, it does significantly reduce the rate of weight gain by adult minnows. The minnows spawned normally in DDEt-saturated water and the percent hatch of the eggs incubated in such water did not differ appreciably from the hatching percentage of control eggs. Although the larvae from eggs incubated in DDEt-saturated water did not differ in appearance from control larvae, they survived poorly in water containing DDEt. Since control larvae also survived poorly in DDEt-saturated water, it is likely that DDEt affects the larvae directly and that mortality among larvae exposed to DDEt is due only slightly or not at all to effects of DDEt upon egg development or the adult reproductive system.

Larval Growth and Survival in Diluted DDEt and TTTB-Saturated Water

Experimental Design

Since DDEt-saturated water proved to be toxic to fathead minnow larvae, tests were undertaken to determine the maximum non-toxic level for DDEt. Similar tests were conducted on dilutions of TTTB-saturated water.

The tests were conducted in 1 x 1 x 2 ft (HWL) tanks. A Mount-Brungs chemical diluter was employed to dilute the saturated toxicant solutions at concentration intervals of 50%. The toxicant was delivered to the initial dilution cell of the diluter with a Harvard infusion pump equipped with a 50 ml syringe containing an acetone solution of the toxicant. The diluter was adjusted to deliver the toxicant solutions to the test tanks at a rate providing five tank volume turnovers per day. The depth of the test solution in each tank was 6 in., the volume was approximately 28ℓ.

The larvae used in these tests were hatched from eggs produced by stock minnows. During the test, the larvae were housed in specially constructed chambers which could be removed from the test tanks for cleaning or whenever a fish census was needed. The chambers were constructed of heavy-walled acrylic tubing with an inside diameter of 4 in. and a length of 7.5 in. Two 2 x 5 in. windows, covered with Nitex screen (40 meshes per inch), were installed around the circumference of the chamber, 1.5 in. from the closed bottom. The test solution from the diluter was delivered to double-drain mixing cells, one located above each chamber. Half of the delivered volume entered directly into the chamber while the other half was delivered to the outer test tank. The larvae were fed on Oregon Moist Trout pellet slurry, *ad libitum*, once a day except on weekends. The length of each test was 30 days. Two tests were conducted on DDEt. TTTB was tested only once.

Data and Discussion

In the first DDEt test, 50 larvae were placed in each chamber. By the end of the test, all fish exposed to the DDEt-saturated solution (50 ppb) had died. Only 4% of the fish exposed to 25 ppb DDEt survived. Survival at DDEt concentrations up to 12 ppb was similar to survival among controls (Table 40). The growth data were examined, but no conclusions could be reached. The mean length of the survivors exposed to 25 and 6 ppb DDEt was slightly lower than the mean lengths of survivors in the other colonies; however, the effect may have been due to space and food limitations as these were the largest colonies. The data from this test indicate a minimum non-toxic DDEt concentration of 12 ppb with the maximum acceptable dose somewhere between 12 and 25 ppb.

TABLE 40

THIRTY-DAY GROWTH AND SURVIVAL OF CONTROL LARVAE EXPOSED TO VARIOUS CONCENTRATIONS OF DDEt-LADEN WATER (Test 1)

			Length, mm	
DDEt (ppb)	Fraction Surviving	% Survival	Mean	Estimated Standard Deviation
50	0/50	0	-	-
25	2/50	4	8.75	0.35
12	31/50	62	8.62	1.21
6	20/50	40	9.58	0.89
3	32/50	64	8.91	1.18
Control	25/50	50	9.73	1.10

The second test on DDEt resulted in slightly different data (Table 41). The data indicate that the MATC of DDEt may be lower than 6 ppb. This compares with an MATC estimate of 12 to 25 ppb based on the data from the first test. Although the mean length of the larvae increased with decreasing concentration, the differences were not significant. A comparison of the mean length for fish reared in 1/2 saturated water (25 ppb) and controls gave a calculated "t" value of 1.43, whereas $t_{.05} = 1.74$.

TABLE 41

THIRTY-DAY GROWTH AND SURVIVAL OF CONTROL LARVAE EXPOSED TO VARIOUS CONCENTRATIONS OF DDEt-LADEN WATER (Test 2)

DDEt (ppb)	Fraction Surviving	% Survival	Mean Peduncle Length and Estimated Standard Deviation (mm)
50	0/25	0	-
25	6/25	24	7.6 ± 1.2
12	6/25	24	7.9 ± 1.2
6	10/25	40	8.0 ± 1.3
Control	14/25	56	8.5 ± 1.7

Fathead minnow larvae, exposed to TTTB solutions of concentrations up to saturation for 30 days, did not appear affected with respect to survival or growth (Table 42).

TABLE 42

THIRTY-DAY GROWTH AND SURVIVAL OF FATHEAD MINNOW LARVAE EXPOSED TO DILUTED TTTB-SATURATED WATER

TTTB (ppb)	Fraction Surviving	% Survival	Length (mm) Mean	Length (mm) Standard Deviation
≤1	25/25	100	9.4	0.9
≤0.5	24/25	96	8.8	1.1
≤0.2	17/25	68	8.2	1.2
≤0.1	24/25	96	9.0	0.9
≤0.06	16/25	64	9.0	0.7
Control	25/25	100	8.7	1.2

In summary, the maximum acceptable DDEt concentration for fathead minnow larvae, based on 30-day larval growth and survival tests, is less than 1/8 the concentration of a saturated aqueous solution of DDEt. This concentration is approximately 6 ppb. This is the lowest concentration which produced a measurable effect on any life stage of the fathead minnow. TTTB saturated water (≤ 1 ppb) has no apparent effect upon growth or survival of fathead minnow larvae.

Chronic Studies with DDEt-Laden Food

Experimental Design

The initial selection of the range of test concentrations for use in a chronic bioassay, where one of the major parameters to be measured is productivity, is often difficult. Ideally, the test concentrations or dosages selected for any bioassay should cover a range that includes toxicant levels which will produce the desired effect or effects. The magnitude of effect should increase in proportion to concentration.

Selection of a concentration range for a chronic bioassay can often be made by carefully examining the dose-response curve constructed from data obtained from acute bioassays performed on the toxicant in question. Generally, the TLm, the slope of the curve and the concentrations which produce no measurable effect are taken into consideration.

Acute toxicity tests performed prior to the chronic tests conducted on DDEt saturated water indicated that DDEt is relatively non-toxic. Although the 96-hr TLm could not be determined, the data indicated that it would probably exceed 6 ppm and that a concentration of 0.10 ppm would probably be a safe concentration for all life stages of the test fish. This early assumption, although erroneous, led to the belief that the maximum acceptable DDEt concentration would exceed saturation, or 0.05 ppm.

For the chronic exposure tests, DDEt concentrations of 5, 1, 0.2 and 0.07 ppm were initially selected. The lowest concentration was thought to be the concentration of DDEt saturated water. This was later revised to 0.05 ppm. Agrimul 70-A was used as a dispersant for DDEt and employed only in the 5, 1 and 0.2 ppm tests. Water containing Agrimul only was used in the control tests. A chemical diluter (Mount and Brungs, Reference 23) was used to dilute and deliver the DDEt-Agrimul suspensions. The DDEt-saturated water did not contain Agrimul and the control medium was in unadulterated laboratory water.

Analysis of the Agrimul suspensions showed only trace amounts of DDEt. Addition of a recirculating pump to stir the Agrimul-DDEt stock solution and increasing the amount of DDEt in the stock solution did not improve the situation. The DDEt saturated water was found to contain 0.05 ppm DDEt consistently.

These problems led to abandoning of the tests involving Agrimul, and the development of another method for testing DDEt levels exceeding saturation. The method of choice was the administration of DDEt to the minnows via their diet. The procedures used in these feeding tests are described below.

The chronic feeding experiments were preceded by a series of acute tests conducted in 1 x 1 x 1 ft. tanks containing 6 in. of water (approximately 14 l). The water in these tanks was changed at a rate of 200 ml per minute or 10 tank volumes per day. For this test, 1, 1.8, 3.2, 5.6 and 10 grams of DDEt in 10 ml of acetone were added to 10 g of Oregon Moist Trout pellets and the solvent evaporated off at room temperature. Food for the control colony was treated in the same manner with solvent only. Analysis of the treated food showed the DDEt content to be within 2% of the calculated level.

The fathead minnows selected for testing were fasted for 24 hours and weighed in groups of 10 before being placed as a group into the test tanks. The DDEt-laden food was provided once daily, except on weekends, at a rate of 5% of body weight. The tests were terminated after 10 days. The dosages and results are shown in Table 43.

TABLE 43

ACUTE TOXICITY OF DDEt TO FATHEAD MINNOWS FED DDEt-LADEN FOOD FOR 10 DAYS

Treatment Group	Mg DDEt/gm fish/day	% DDEt in diet	Appearance of fish
Control	0	0	Normal
1	4.6	9.1	Normal
2	7.6	15.2	Normal
3	12.1	24.3	Emaciated
4	17.8	35.8	Emaciated
5	25.0	50.0	Emaciated

All but one of the test fish survived the 10 day test. However, although the fish readily consumed the treated food initially, those receiving diets containing 24.3% or more DDEt began to eat less food after three days. Fish in these groups were noticeably emaciated by the seventh day and one fish in the colony receiving the 24.3% diet died. Anorexia and emaciation became more and more evident with time. Fish receiving less than 24.3% DDEt in their diet readily consumed food throughout the test and did not appear affected. On the basis of this test, the maximum dosage selected for the chronic test was 2 g DDEt per 100 g of food or approximately a 2% diet (i.e., ca 10% of dose giving an effect in seven days or less).

The long term tests were initiated on June 16, 1971. Test procedures and conditions were the same as in the long term study on DDEt saturated water with a few exceptions. Instead of delivering the test water to the larval tanks via mixing cells, the water was fed directly into each larval tank by means of individual feedlines. As before, the water passed through the larval tanks and into the adult tanks before being sewered.

The initial day length was set at 13.5 hrs, corresponding to mean day length for April 1 on the attached photoperiod schedule (Appendix). After the maximum day length on the schedule was reached, it was further increased to 16 hrs and maintained at this level for the remainder of the study.

The test fish were selected from the minnow stock purchased on May 18, 1971. The fish were individually measured for length (fork lengths) and then weighed in groups of five. The groups were assigned to the test tanks by random stratified assortment until each tank contained 15 fish. The 10 tanks for the adult fish were than randomly assigned numbers and placed on the tank racks serially to minimize possible effects caused by tank location.

The DDEt-laden food was prepared by adding 0.002, 0.02, 0.20 and 2.0 g of DDEt in 100 ml of acetone to 100 g of Oregon Moist Trout pellets and evaporating the solvent at room temperature. Food for the control colonies was prepared in the same manner with the same amount of solvent added. The food was kept frozen, but thawed each day before use.

The fish were fed once daily, except on weekends, at a rate of 5% of body weight. To establish the food-weight relationship, the fish in each tank were weighed as a group every two weeks. The dosages were 0, 0.001, 0.01, 0.10 and 0.98 mg DDEt per g fish per day. These dosages are equivalent to 0.02, 0.2, 2.0 and 20 mg DDEt per g food per day. Food left uneaten after 4 hr was siphoned out and discarded. When the fish began to spawn, the weighing schedule was abandoned so as not to disturb the fish and food was provided once a day _ad libitum_.

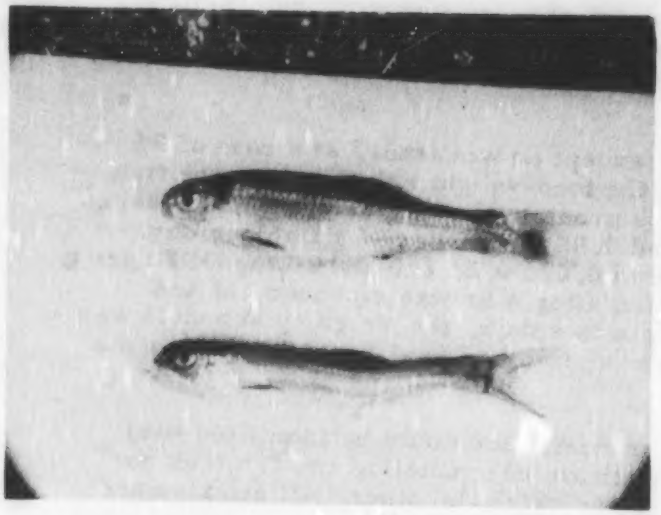
When the fish reached sexual maturity and could be identified with respect to sex, a screened partition was installed in each tank so that the volume of one section was twice the other. All females and three males were permitted to remain in the larger section and were provided with three spawning tiles. The rest of the males were placed in the smaller section. Males with the females were replaced whenever any of these males died.

Data and Discussion

During the first 10 weeks all adult fish receiving 20 mg DDEt per g of food developed symptoms of a syndrome known as the "benzene bends" and died. The symptoms started with refusal of food, followed by progressive emaciation. In the later stages, the fish developed dorso-ventral spinal curvature and often swam erratically near the bottom of the tank at an angle varying from 45 to almost 90 degrees from horizontal. The terminal stage was marked by extreme emaciation and feebleness. At this stage the fish lay on its side on the bottom of the tank. Except for opercular movement, the fish generally lay motionless until death. Figure 2 compares the appearance of a typically affected fish with that of an unaffected fish.

Mortality among fish in the remaining colonies varied considerably between duplicate tests (Table 44) and appeared unrelated to DDEt dosage. Although typical toxic symptoms developed in some of the fish, the occurrence of these symptoms was not consistent. The two dead fish recorded for group 1 (0.02 mg DDEt) and all six dead fish from both of the groups which received a dosage of 0.2 mg DDEt per g food per day developed definite toxic symptoms. These fish represented only 25.8% of the total number of fish dying during the 28- week test.

Interestingly, of the 31 dead fish recorded (excluding those which had received the highest dose) 4% were males. In terms of sex, 1% of the remaining males and only 7% of the remaining females died. Low survival among males was also observed in similar tests with TTTB laden food.


Control (A)
DDEt -Exposed (B)

Fish A - Control
Fork Length 62mm
Weight 3.33g

Fish B - DDEt -Exposed
Fed 980mg DDEt /kg Body Weight/Day for 50 Days
Fork Length 55mm
Weight 1.51g

Figure 2. EFFECT OF FEEDING HIGH DOSE RATE OF DDET ON FATHEAD MINNOW

TABLE 44

MORTALITY OF FATHEAD MINNOWS FED DIFFERENT DOSAGES OF DDEt IN THEIR DIET

		Females			Males			
Mg DDEt/gm Food	Group	Initial	Final	Dead	Initial	Final	Dead	Total Dead(%)
Control	1	13	6	7	2	2	0	42
Control	2	11	10	1	4	3	1	14
0.02	1	12	10	2	3	3	0	14
0.02	2	12	10	2	3	2	1	20
0.20	1	12	11	2	3	3	0	33
0.20	2	10	6	4	5	5	0	27
2.0	1	11	10	1	4	4	0	7
2.0	2	13	3	10	2	2	0	7
20.0	1	11	11	11	4	0	4	100
20.0	2	12	12	12	3	0	4	100

The reason for low male survival is not known. The amount of DDEt in their diet did not appear responsible. One explanation is that the population may have contained a large percentage of second season males. According to Markus (Reference 33), 80 to 85% of the first year spawners die after spawning. The carryovers almost invariably die after spawning the second time. The test fish for this study and the study on TTTB laden food were selected from the minnow stock acquired in May. Why the May-acquired stock contained 78% males and the January stock contained only 48% males is beyond the scope of this study.

During the first 12 weeks before spawning commenced, the control colonies gained an average of 0.26 g per fish per two week interval. This average was calculated from pooled data from both duplicate colonies.

Colonies which received 0.02, 0.20 and 2.0 mg DDEt diets gained weight at an average rate of 0.25, 0.26 and 0.21 g per fish. Fish fed 20 mg DDEt per g food were weighed twice a month for only eight weeks. The bi-monthly growth rate was only 0.017 g.

In general, the total gain in weight was somewhat similar for all groups except those on the 20 mg DDEt diet and possible those on the 2.0 mg DDEt diet (Table 45). The latter gained only 74% as much as controls and the former gained only 3.4% as much as controls. In all groups except those on the 20 mg DDEt diet, the mean gain in length was similar.

TABLE 45

GROWTH OF FATHEAD MINNOWS FED DDEt-LADEN FOOD FOR 28 WEEKS

mg DDEt/g Food	Group	Mean individual fork length (mm) Initial	Final	ΔLength	Mean individual weight (g) Initial	Final	ΔWeight
Control	1	54.8	67.8	13.0	2.2	5.1	2.9
Control	2	53.1	65.8	12.7	1.9	4.4	2.5
0.02	1	51.5	66.1	14.6	1.8	4.2	2.4
0.02	2	51.2	65.0	13.8	1.7	4.3	2.6
0.20	1	55.1	68.5	13.4	2.1	5.4	3.3
0.20	2	52.9	65.6	12.7	1.7	4.2	2.5
2.00	1	55.3	67.8	12.5	2.2	4.1	1.9
2.00	2	51.9	65.8	13.9	1.7	3.9	2.2
20.0	1	52.8	-	-	1.9	2.0	0.1*
20.0	2	54.6	-	-	2.1	2.2	0.1*

* 8 weeks

The minnows started to spawn on September 13 and continued until January 19, a period of about four months. The sex ratio and data on spawning and egg hatching success for the 10 colonies are listed in Table 46. Except for the two colonies which were fed 20 mg DDEt per g food, there was no consistent relationship between the amount of DDEt in the diet and the number of spawns per female. Although one of the control colonies produced 7.5 spawns per female, its duplicate produced only 0.75 spawns per female, a performance not appreciably different from that of any other colony from which spawns were obtained.

Hatching success of the eggs produced by colonies fed 2 mg DDEt per g of food was significantly lower than the hatching success of eggs produced by controls. A Students "t" test on the difference between the mean percent hatch based upon pooled data, gave a t_{calc} of 2.53, whereas $t_{0.05}$ is 1.77. Hatching success of the two other colonies which produced eggs was similar to that of controls.

The fry from all colonies normally hatched within five to seven days after they were transferred to the incubation cups. There was no noticeable difference in the appearance of the fry upon hatching. Hatching percentages varied considerably and there was no appreciable difference in the mean length of the fish after 30 days (Table 47). Due to the paucity of data, the effect of DDEt on larval growth and survival cannot be evaluated.

TABLE 46

EGG PRODUCTION AND HATCHABILITY BY FATHEAD MINNOWS FED DDEt-LADEN FOOD

			mg DDEt per g Food							
	Control		0.02		0.20		2.0		20	
Group:	1	2	1	2	1	2	1	2	1	2
No. of males	3	3	3	3	3	3	3	3	0	0
No. of females	2	4	3	3	3	5	4	2	0	0
No. of spawns	15	3	4	0	1	2	0	4	0	0
Spawns/female	7.5	0.75	1.33	0	0.33	0.40	0	2.0	0	0
Av. eggs/spawn	119.9	108.7	157.0	0	393	170.0	0	67.8	0	0
Eggs produced Total	1799	326	628	0	393	340	0	271	0	0
Percent hatch (N)	85.8(8)	90.0(3)	96.0(3)	-	-	92.0(3)	-	76.6(3)	0	0

TABLE 47

PERCENT SURVIVAL AND MEAN LENGTH OF FATHEAD MINNOW LARVAE FED DDEt-LADEN FOOD

Test Number	mg DDEt per g food				
	0	0.02	0.20	2.0	20.0
1	24.0	0	39.0	13.7	0
2	2.9	42.0	32.0	0	0
3	45.7	14.0	0		
4	30.2				
5	40.9				
6	14.9				
7	40.0				
8	17.5				
Mean Survival, %	27.0	18.7	23.3	6.8	0
Mean length, mm	9.1	8.9	10.0	9.6	-

Administered *per os,* DDEt appears considerably less toxic to fish than DDT administered in the same manner. Cutthroat trout, fed a pelleted diet containing DDT at a rate of 1 mg DDT per kg every seven days showed a significantly high mortality rate compared to controls after four months of feeding (Reference 34). The calculated daily rate of DDT intake would be 0.183 mg per kg (1 mg/seven days). In the present study, fathead minnows on the 0.02 mg DDEt per g food were fed at a rate of 10 mg per kg body weight per day during the first three months and possibly more subsequently when they were fed *ad libitum.* At this dosage rate, the minnows were not affected with respect to growth survival and egg production. The 2.0 mg DDEt per g food diet, fed at a rate of at least 100 mg DDEt per kilogram body weight per day, reduced egg hatchability significantly in fathead minnows and probably reduced growth somewhat. Neither dosages affected survival.

Species differences could account for some of the difference in toxicity; however, it is unlikely that the 50-500-fold greater toxicity of DDT could be due to species differences alone. The 96-hr TLm of DDT to fathead minnows, goldfish, guppies and bluegills range only from 0.021 to 0.056 ppm (Reference 27), and the 96 hr TLm for rainbow trout range from 0.024 to 0.074 ppm (Reference 35). For these five fish species the highest TLm concentration is no more than four times the lowest.

Chronic Study on TTTB-Laden Food

Experimental Design

The procedures employed in this study were the same as those used in the chronic study on DDEt-laden food. The test tanks, however, were 1 x 1 x 3 ft (HWL) tanks with two sections 12 x 10 x 6 in. partitioned off with 40 mesh Nitex screen to serve as larval rearing areas. These tanks permitted the adults and larvae to be maintained in one tank and conserved a considerable amount of laboratory space.

Since TTTB is only sparingly soluble in acetone, the treated food was prepared by dissolving 0.002, 0.02, 0.20 and 2.0 g of TTTB in 100 ml of tetrahydrofuran (THF), mixing the solution with 100 g of Oregon Moist Trout pellets and evaporating the solvent at room temperature. Preliminary tests showed that 555 mg THF per liter was not toxic to fathead minnows over a period of 96 hours and that food containing as much as 24% TTTB had no effect upon fathead minnows over a period of 10 days.

The study was initiated on June 16, 1971 with minnows purchased on May 18, 1971. The initial number of fish per duplicate tank was 15.

Data and Discussion

During the 28 week test, mortality was similar for all treatment groups (Table 48). A total of 48 fish died during the test; 94% of these were males. The male population suffered 39% mortality and the female population only 9%. None of the dead fish showed symptoms of the "benzene bends." Of the 48 total, 23 or about 48% died from an apparent bacterial infection. The causative agent was investigated but not identified. Tetracycline treatment for 3-4 days was effective in controlling the infection. Subsequent deaths did not appear dose-dependent.

TABLE 48

MORTALITY OF FATHEAD MINNOWS FED DIFFERENT DOSAGES OF TTTB IN THEIR DIET

mg TTTB/ g food	Group	Initial	Final	Dead	Initial	Final	Dead	Total Dead(%)
Control	1	11	7	4	4	3	1	33
Control	2	13	3	10	2	2	0	68
0.02	1	11	7	4	4	4	0	28
0.02	2	11	4	7	4	3	1	53
0.20	1	13	9	4	2	2	0	27
0.20	2	12	12	0	3	3	0	0
2.0	1	13	8	5	2	2	0	33
2.0	2	13	7	6	2	2	0	40
20.0	1	10	7	3	5	4	1	27
20.0	2	9	7	2	6	6	0	14

The fish began to spawn on August 18. Up to this date the fish were weighed bi-monthly. The mean increase in individual weight, calculated from pooled data was 0.29, 0.21, 0.25, 0.26 and 0.25 g, respectively, for controls and for colonies receiving 0.02, 0.2, 2.0 and 20 mg TTTB per g food per day. The slightly higher growth rate of controls during this period was not carried through to the end of the test. Fish in all colonies showed similar overall length and weight gains (Table 49).

TABLE 49

GROWTH OF FATHEAD MINNOWS FED TTTB-LADEN FOOD FOR 28 WEEKS

mg TTTB/ g food	Group	Mean fork length (mm)			Mean individual weight (g)		
		Initial	Final	ΔLength	Initial	Final	ΔWeight
Control	1	52.2	65.0	12.8	1.7	4.1	2.4
Control	2	53.9	65.2	11.3	1.7	4.7	3.0
0.02	1	53.5	65.9	12.4	1.8	3.9	2.1
0.02	2	53.9	66.6	12.7	1.8	4.5	2.7
0.20	1	53.1	66.9	13.8	1.7	4.4	2.7
0.20	2	54.0	66.9	12.9	2.0	4.5	2.5
2.0	1	54.1	67.5	13.4	1.9	4.6	2.7
2.0	2	54.5	70.9	16.4	1.9	5.3	3.4
20.0	1	56.1	66.7	10.6	1.9	4.3	2.4
20.0	2	55.1	67.4	12.3	2.1	4.3	2.2

The number of spawns per female was inconsistent. The two colonies on the 2 mg DDEt diet spawned a total of 12 times for spawn per female records of 4 and 2. One control colony spawned three times per female but all other colonies spawned no more than 0.5 times per female (Table 50).

TABLE 50

EGG PRODUCTION AND HATCHABILITY BY FATHEAD MINNOWS FED TTTB-LADEN FOOD

	mg TTTB per g food									
	Control		0.02		0.2		2.0		20	
	1	2	1	2	1	2	1	2	1	2
No. of males	3	3	3	3	3	3	3	3	3	3
No. of females	4	2	4	4	2	3	2	2	5	6
No. of spawns	1	6	2	1	0	1	8	4	0	1
Spawns/female	0.25	3.0	0.50	0.25	0	0.33	4.0	2.0	0	0.17
Mean eggs/spawn	40.0	321.0	14.5	30.0	0	83	148.0	90.2	0	22
Total eggs	40	1926	29	30	0	83	1184	361	0	22
Percent hatch (N)	25(1)	71.6(6)	100(2)	98(1)	-	48(1)	63.8(8)	85(4)	-	90(1)

Hatching percentages also varied considerably (Table 50). In general, the hatching percentage of eggs from fish fed TTTB-laden food was no worse than the hatching percentage of eggs from controls.

The data on growth and survival of larvae fed the same diet as the parental fish are shown in Table 51. Very little data was obtained on this phase of testing as none of the treatment groups produced a sufficient number of spawns. The data obtained were highly variable. None of the individuals in five of the larval groups tested at 0.2 mg TTTB/g food and higher survived the 30 day exposure period. While this may indicate a possible effect of TTTB, one of the groups tested at 2.0 mg TTTB/g food showed 84.4% survival which was higher than that of any other group in the entire test. Also, survival of groups from tank 2, 2.0 mg TTTB/g food, compared favorably with survival of control groups. Hence, the effect of TTTB on the survival of fathead minnow larvae is questionable; the tests bear repeating.

In summary, the data indicate that a diet of Oregon Moist Trout pellets containing up to 2 g DDEt per 100 g of pellets has no effect upon the growth, survival, or egg production of fathead minnows. Data on egg hatchability and larval growth and survival was insufficient for statistical treatment; hence, no conclusions can be made on the effects of TTTB-laden food on these parameters.

TABLE 51

THIRTY-DAY GROWTH AND SURVIVAL OF FATHEAD MINNOW LARVAE FED TTTB-LADEN FOOD, %

	Control		mg TTTB per g food							
			0.02		0.20		2.0		20.0	
Group:	1	2	1	2	1	2	1	2	1	2
	No spawn	24.0	28.0	16.0	No spawn	0	0	18.0	No spawn	0
		44.4	36.4				0	36.2		
		26.0					0	28.4		
		46.0					4.0			
		42.9					84.4			
Mean survival, %		37	32	16	-	0	18	28	-	0
Mean length, mm		9.0	8.4	8.8	-	-	8.7	9.0		

SECTION VII

ANALYSIS OF STUDIES

The studies described in the preceding sections represent the initial development of a concept of controlled destruction of field-applied pesticides, such as DDT. The feasibility of the overall concept was shown in earlier studies (Reference 1). Two areas were analyzed: the basic chemical reactions involved in degrading DDT, and the expected safety of the products to various life forms.

Both copper-catalyzed aluminum and copper-catalyzed iron reductants appear to give sufficiently rapid and extensive degradation of DDT to be useful for field degradation. The decomposition with the Al·Cu system requires 24 hr at 25°C, and 4 hrs at 40° (simulating summertime conditions in warmer climates), while 1-2 weeks at 25°C and 8 hrs at 40°C is needed for decomposition by the Fe·Cu system. Although neither system gives as rapid decomposition as the copper-catalyzed zinc reductant, the systems are sufficiently rapid for destruction of field-applied DDT.

The use of copper-catalyzed aluminum or iron overcomes a potential problem attendant to the use of catalyzed zinc reductant. The reaction involving zinc produces 3 equivalents of zinc ion per equivalent of DDT reduced. This is a drawback since zinc ion in sufficient quantity is known to be toxic to fish. Sources cited in Reference 1 indicate that zinc ion concentrations as low as 0.01 mg/l may cause deleterious effects to certain fish species; with other fish, the median tolerance limit may be as high as 35 mg/l of zinc ion. However, the use of catalyzed iron or aluminum appears to obviate this problem, since the iron or aluminum ions in reasonable quantities are apparently not toxic to fish. Importantly too, only one equivalent of iron or aluminum is required theoretically per equivalent of DDT, so that the amount of dissolved metal is reduced significantly. The economic advantage of reduced metal consumption is also important. The comparison of theoretical metal usage and cost is given in Table 52.

TABLE 52

THEORETICAL METAL USAGE AND REDUCTANT COST FOR REDUCTIVE DEGRADATION OF DDT

Reductant	Al·Cu Couple	Fe·Cu Couple	Zn·Cu Couple
Theoretical: equiv. reductant/mol DDT	1	1	3
Theoretical: lb metal ion/lb DDT	0.025	0.052	0.28
Theoretical Reductant cost* cents/lb DDT	1.0	0.2	5.5

* Based on Zn dust at \$0.20/lb, Al powder at \$0.40/lb, Fe powder at \$0.04/lb

The practical deployment of a reductively degradable form of DDT apparently requires a source of acidity for the reaction. Requirements for the avoidance of phytotoxic damage as well as economy of reagents suggests that the amount of acid employed should be minimal. The use of an acid which exists in solid state, and which might be placed in close proximity to the reactants (DDT and reductant) appears to be a suitable means for carrying out the reaction.

The discovery that moderately strong acids, such as sulfamic, are preferred is important to the development of a practical system. The difficulties in removing water of crystallization and in an early reaction of aqueous acid and reductant appears readily solvable with proper choice of non-aqueous solvent, or in using a technique such as spray drying for application of the acid. Minimum acid requirements for effective reaction have not been established, but the cost appears to be low. The quantity of acid employed in early tests was 15 millimoles of acid per gram of DDT. The cost of this quantity of sulfamic acid would be about \$0.21 per pound of DDT; again, it should be emphasized that minimum amounts of acid for effective action have not been determined and it is expected that the quantity and cost can be appreciably reduced. The dissemination of 1.5 pounds of acid/acre (assuming 1 lb/acre of DDT and 1.5 lbs sulfamic acid/lb DDT or 15 millimoles/g DDT) would appear to offer no significant problem with respect to modifying soil characteristics.

The results involving tests of the integrated particle concept in which a fine reductant particle was overlaid with the solid acid and the whole covered with DDT, leads strongly to the conclusion that the concept is both practical and workable. Only moisture is required to initiate the reaction. The inclusion of a slowly dissolving, permeating, eroding, or rupturing membrane between the acid coat and the pesticide layer, to provide the requisite delay to allow for pest control action, would result in the desired controlled, self-destructing form of the pesticide. Although the reported tests involved the catalyzed zinc reductant system, all evidence points to equivalent degradation employing the catalyzed aluminum and iron systems.

It would be important in considering this concept to examine the properties of the integrated particle on dissemination. It has been shown in an earlier analysis that 5-20 µm particle size in aerosol spray appears desirable on the basis of experience, as well as aerodynamic capture, settling velocity, and related factors (Reference 1). While it may be considered that the pest control effectiveness of the integrated particle is not affected by the process of forming the particle, since the DDT is on the outside of the particle in unmodified form, close examination of the concept is warranted. Important to the analysis is the consideration of the mean interparticle distance in uniformly dispersed particles, since too great a distance would reduce the probability of effective pest control action. Calculations can then show the number of particles per unit area, and the mean interparticle distances, assuming uniform distribution. This calculation (Table 52) is based upon the use of 4 lbs of reductant and 1 lb of DDT per acre.

TABLE 53

CALCULATED EFFECT OF PARTICLE SIZE ON THE MEAN PARTICLE-PARTICLE DISTANCE FOR Al·Cu AND Fe·Cu REDUCTANTS, INTEGRATED SELF-DESTRUCTING PESTICIDE CONCEPT

Reductant	Al·Cu		Fe·Cu	
Particle Size µm	Particles/cm^2	Mean Particle-Particle Dist. µm	Particle/cm^2	Mean Particle-Particle Dist. µm
5	2.5×10^5	20	8.9×10^4	33
10	3.2×10^4	56	1.1×10^4	95
20	4.0×10^3	160	1.4×10^3	450

These distances appear sufficiently small so that minimal movement by the pest will assure contact with the pesticide. Hence, pest control action should be highly effective. As shown previously, particles of 5-20 µm mean diameter should be readily handled by conventional spray or dusting equipment.

The fate of the degradation products in the environment also requires consideration.

The copper-catalyzed aluminum reduction of DDT leads to the production of TTTB and DDA as products, while the catalyzed iron reduction leads mainly to the formation of TTTB. The product TTTB is insoluble in water (1 ppb or less solubility at 40°C), and the solubility in the fat triolein is 50-fold less than DDT. Hence, the material tends to remain in the environment as an inert solid, rather than being dispersed in land runoff, or in being transmitted in life forms through fat-solubility, as DDT. Limited hydrolytic stability tests indicated an intrinsic stability of the material, and other tests indicated resistance to further chemical reaction. Attempts to measure the vapor pressure were not successful, but the fact that the material is a high melting solid (268-270°C melting point) suggests that the vapor pressure is very low and evaporative losses would be negligible.

The product DDEt, obtained from the catalyzed zinc reduction of DDT, is readily dispersed, however. The solubility in water is about 20-fold greater than DDT, and the solubility in the fat triolein is about 2-1/2-fold greater than DDT. The vapor pressure of DDEt is about 80-fold greater than DDT, and evaporative loss of the product from the field was calculated to be significant. Indeed, long term laboratory experiments of particle degradation have frequently led to lower than expected product recovery; DDEt evaporation is believed responsible for the apparently low recovery of the product.

The toxicity studies also showed a significant difference between the two basic reductive degradation techniques; Al·Cu or Fe·Cu reduction leading to TTTB (and DDA), or Zn·Cu reduction producing mainly DDEt.

No adverse effects were noted with TTTB, on the basis of long term chronic effects, or 96 hr acute toxicity tests with fathead minnows. The growth and survival of adults over a 7 month period resembled control data. The egg production rate, hatchability and growth and survival of freshly-hatched fry were all statistically similar to those of unexposed control fathead minnow colonies. Feed tests in which about 0.1% of the diet consisted of TTTB (980 mg/kg body weight/day) also produced no evident toxic effects.

Acute testing with DDEt, in which fathead minnows were exposed to suspensions containing initially as much as 100 ppm DDEt, as well as tests with saturated solutions (0.05 ppm), produced no toxic effects. Similarly, 7 month tests of the growth and survival of adult fathead minnows, egg production, and hatchability appeared similar for control groups and colonies exposed to DDEt-saturated water. However, a significant mortality of freshly hatched fry exposed to DDEt-saturated water was noted, and the concentration of DDEt had to be reduced to about 6 ppb before fry mortality ceased. Thus, the maximum acceptable toxicant concentration appears to be about 6 ppb. Tests in which DDEt was added to the diet indicated that levels of DDEt greater than 10 mg/kg body weight/day led to toxic effects. At high concentrations of DDEt, emaciation and dorso-ventral spinal curvature was noted. Grossly exposed fish were observed to swim erractically before succumbing. The symptoms are understood to be similar to the "benzene bends" described for fish exposed to the chlorinated pesticide lindane.

In summary, a number of advantages of the catalyzed aluminum or catalyzed iron reductant systems are shown over the catalyzed zinc system. Foremost among these is the lack of any discernible toxic effect on fish from either short term acute testing or long term chronic testing. The reaction appears to proceed smoothly at reasonable rates to a product shown to be inert to the environment under the conditions tested.

The data indicate the reaction can be carried out in a practical way, and means are suggested for the application of the concept. The results of this initial developmental study are believed to strongly fortify the conclusions of the feasibility study that the technique is a practical and useful one. Further, development and implementation of the concept is strongly recommended.

SECTION VIII

ACKNOWLEDGEMENTS

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The help of Mr. John Eston of the National Water Quality Laboratory of the Environmental Protection Agency, Duluth, Minnesota, has been appreciated. His suggestions relative to the dispersal of toxicants, and in the biology and rearing of fathead minnows have been very helpful.

The assistance of California Fish and Game biologists in enabling the rearing of disease-free fish is also acknowledged. The contributions of Mr. Harold Wolf, Fish and Game Pathologist, and Mr. William Richardson, Inland Fisheries Section, are particularly noted.

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SECTION IX

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SECTION X

PATENTS AND PUBLICATIONS

Patent applications will be prepared for significant findings if not covered by the applications filed under the preceding program, Contract 14-12-596. Technical papers describing findings on the chemistry of DDT and toxic testing of products are planned.

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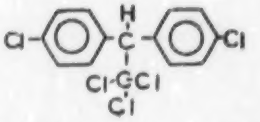
SECTION XI

GLOSSARY

CHEMICAL FORMULAS OF PESTICIDES AND DEGRADATION PRODUCTS

DDT*



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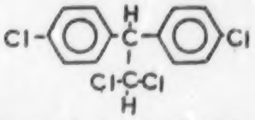
DDD* (TDE)



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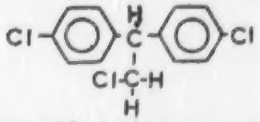

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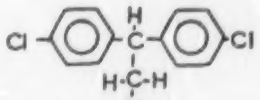
DDEt



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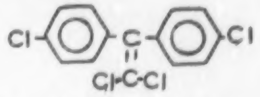
DDE



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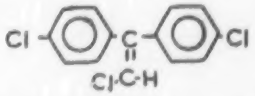

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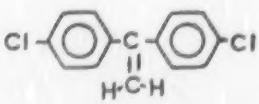

1, 1-bis(p-chlorophenyl) ethylene

* Coding of compounds used by Menzies, "Metabolism of Pesticides" (Reference 3)

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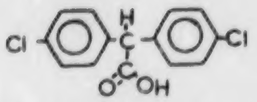
GLOSSARY

CHEMICAL FORMULAS OF PESTICIDES AND DEGRADATION PRODUCTS

DDA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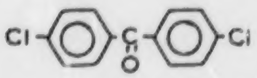
C
C
O
OH
Cl

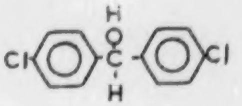
bis(p-chlorophenyl)acetic acid

DBP



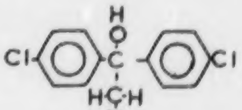
4, 4'-dichlorobenzophenone

DBH



4, 4'-dichlorobenzhydrol

DMC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H
Cl

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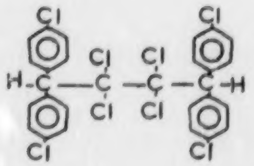
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Cl
C-H
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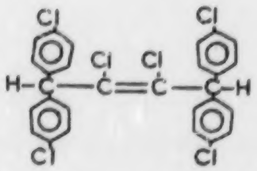
TTDB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C
Cl
C-H
Cl

1, 1, 4, 4-tetra(p-chlorophenyl)-2, 3'-dichlorobutene-2

DEFINITION OF TOXICOLOGICAL TERMS

96-hr TLm - The concentration, estimated from data obtained via acute toxicity experiments, lethal to 50 percent of the test species during a 96-hr exposure period.

MATC - The maximum acceptable toxicant concentration. A term proposed by Mount and Stephan (Reference 24) to denote the toxicant concentration having no adverse effect upon any stage in the life cycle of the test species.

Application factor - A constant obtained by dividing the MATC determined empirically for a given test species by the 96-hr TLm determined for the same species.

SECTION XII

APPENDIX

Test (Evansville, Indiana) Photoperiod

For Fathead Minnow Chronic Testing

Dawn to Dusk Time	Date	Day Length (hour and minute)	
6:00 - 4:45)	Dec. 1	10:45)	
6:00 - 4:30)	15	10:30)	
6:00 - 4:30)	Jan. 1	10:30)	
6:00 - 4:45)	15	10:45)	
6:00 - 5:15)	Feb. 1	11:15)	5-month pre-spawning growth period
6:00 - 5:45)	15	11:45)	
6:00 - 6:15)	Mar. 1	12:15)	
6:00 - 7:00)	15 15	13:00)	
6:00 - 7:00)	Apr. 1	13:30)	
6:00 - 8:15)	15	14:15)	
6:00 - 8:45)	May 1	14:45)	
6:00 - 9:15)	15	15:15)	
6:00 - 9:30)	June 1	15:30)	4-month spawning period
6:00 - 9:45)	15	15:45)	
6:00 - 9:45)	July 1	15:45)	
6:00 - 9:30)	15	15:30)	
6:00 - 9:00)	Aug. 1	15:00)	
6:00 - 8:30	15	14:30)	
6:00 - 8:00)	Sep. 1	14:00)	
6:00 - 7:30)	15	13:30)	
6:00 - 6:45)	Oct. 1	12:45)	Post spawning period
6:00 - 6:15)	15	12:15)	
6:00 - 5:30)	Nov. 1	11:30)	
6:00 - 5:00)	15	11:00)	

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